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Abstract: Hydrophylax bahuvistara, a new species of fungoid frog, is described from peninsular India. It can be separated from its congeners based on a combination of characters including wider head, outline of snout in dorsal view truncated, finger and toe tips without lateroventral groove, foot moderately webbed, metatarsals of 4th and 5th toes closely set, outer metatarsal tubercle small, foot length less than or equal to half of snout vent length, dorsal parts of shank without glandular folds and sparse horny spinules, and heels touch each other when the legs are folded at right angles to the body. Genetically, H. bahuvistara forms a monophyletic group with H. malabaricus as a sister clade separated by a raw distance of 4.0 to 4.5% in the 16s rRNA gene. Morphometrically, H. bahuvistara forms a significantly different cluster from H. malabaricus and H. gracilis in Discriminant Analysis.

Keywords: Anura, molecular taxonomy, multivariate analysis, taxonomy.

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Hydrophylax bahuvistara, a new species of fungoid frog (Amphibia: Ranidae) from peninsular India

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INTRODUCTION

Fitzinger (1843) raised genus Hydrophylax with Rana malabarica Tschudi, 1838; as the type species by original designation. However, Günther (1859) implied synonymy of Hydrophylax to Rana Linnaeus, 1758, and Günther (1864) implied synonymy of Hydrophylax to Hylarana (an incorrect spelling of the genus Hylarana Tschudi, 1838) by placing the type species under the respective genera. Boulenger (1920) considered Rana malabarica as member of the subgenus Rana. Dubois (1992) considered Hydrophylax as a subgenus under the subsection Hydrophylax, section Hylarana of genus Rana and considered two valid species R. malabarica and R. galamensis Dumeril & Bibron, 1841. Frost et al. (2006) considered Hydrophylax as a valid genus and placed 21 species, including R. malabarica in this genus. Che et al. (2007) synonymized Hydrophylax to Hylarana and the same was followed by Biju et al. (2014). Oliver et al. (2015) resurrected Hydrophylax based on genetic analysis and considered three species, viz., H. gracilis (Gravenhorst, 1829), H. leptoglossa (Cope, 1868) and H. malabaricus, under this genus.

The genus Hydrophylax is diagnosed based on a combination of characters including presence of a postocular mask, robust body, rear of thighs with strong vermiculations, large rictal gland, prominent humeral gland, and ventrolateral grooves sometimes absent on finger 1 (Oliver et al. 2015). Currently recognized species in the genus are widely distributed in Asia and are known from Sri Lanka, India, Bangladesh, southern Myanmar and western Thailand (Frost 2015; Oliver et al. 2015).

In a recent review of golden-backed frogs from the Western Ghats of India and Sri Lanka, Biju et al. (2014) considered Rana malabarica as a member of the genus Hylarana. They suggested that this widespread species, which is spread across peninsular India, harbors two genetically distinct haplogroups, one restricted to the Western Ghats of Kerala and Tamil Nadu and the other distributed in the Western Ghats of Karnataka, Goa and Maharashtra and extending as far as Madhya Pradesh in central India. While the specimens from Western Ghats of Kerala and Tamil Nadu could be attributed to Hydrophylax malabaricus sensu stricto, Biju et al. (2014) refrained from describing the haplogroup north of Kerala as a distinct species owing to limited data and absence of diagnostic characters for the separation of the haplogroups, and considered it as ‘Hylarana malabarica’ haplogroup 1.’

Here we report the description of a new species of fungoid frog Hydrophylax bahuvistara, considered as ‘Hylarana malabarica’ haplogroup 1’ by Biju et al. (2014), based on the study of type and topotypic material of Hydrophylax malabaricus and specimens collected from a wide range within peninsular India from Karnataka, Goa, Maharashtra and Madhya Pradesh states. We show that the new species can be delineated from typical H. malabaricus based on both genetic and multivariate morphometric analysis, and is diagnosable based on morphological combination of characters.

MATERIALS AND METHODS

Specimen collection

Specimens of the new species were collected in India from Goa State and Pune, Raigad, Sindhudurg, Ratnagiri, Thane, Chandardpur and Satara districts of Maharashtra State. The specimens were collected from a variety of habitats, including roadside paddy fields and pools, grasslands, temporary rainwater pools on plateaus, lake shores and stream banks in semi-evergreen forests. A total of 20 specimens (Table 2) were collected and not more than two specimens were collected from each locality except for the type locality at Tamhini, where four specimens were collected. Four specimens of H. malabaricus were collected from the main campus of the Kerala Agricultural University, Thrissur, Kerala, India. These specimens were considered as topotypes as the area is within the type locality in Malabar mentioned in the original description, and specimens agree in morphology with the lectotype of H. malabaricus. Specimens were preserved in 70% ethanol with 5% glycerol.

Museum details

Specimens collected in the present study are deposited in the museum collection of the Bombay Natural History Society (BNHS), Mumbai; the Zoological Survey of India, Western Regional Center (ZSI-WRC), Pune; the Wildlife Information Liaison Development (WILD), Coimbatore; the Abasaheb Garware College, Zoology Research Laboratory (AGCZRL), Pune and the Kerala Agricultural University Natural History Museum (KAUNHM), Thrissur, Kerala. Type material of Hydrophylax malabaricus was studied from Muséum National d’histoire Naturelle, Paris (MNHN). Photographs of Hydrophylax leptoglossa syntypes were obtained from Museum of Comparative Zoology (MCZ), Harvard University. Photographs of Hydrophylax gracilis were obtained from Natural History Museum (UWZM), Wroclaw University.
Morphometry

Measurements were taken to the nearest 0.1 mm using a digital caliper (Ocean Premium measuring instruments), as defined in Biju et al. (2014) and include: snout-vent length (SVL); head width (HW); head length (HL); tympanum diameter (TYD); snout length (SL); inter upper eyelid width (IUE); maximum upper eyelid width (UEW); eye to nostril length (EN); snout to nostril length (SN); eye length (EL); forelimb length (FAL); hand length (HAL); thigh length (TL); shank length (SHL); foot length (FOL).

Statistical analysis

All the measurements showed a positive linear relationship with SVL. Thus to remove the effect of size, morphometric data were normalized by expressing measurements as a percentage of SVL. Multivariate normality of the data was checked using the Doornik & Hansen (2008) omnibus. Discriminant Analysis (DA) performed to understand whether related species of *Hydrophylax* form significantly different clusters (Huberty & Olejnik 2006). Pillai’s trace statistic was used to test the null hypothesis that the mean vectors of different clusters are equal (Harris 2001). Mahalanobis distances (Harris 2001) between pair of individuals were calculated and used for computing Fisher’s distances (distance between the centroids of the clusters, divided by the sum of their standard deviations) between clusters to determine if the clusters were significantly different. Statistical analysis was performed in PAST 3.0 (Hammer et al. 2001).

Molecular analysis

Thigh muscle tissue was harvested from twelve specimens of the new species (11 marked by asterisk in Table 1 and WILD-13-AMP-011) and four specimens of topotypic *H. malabaricus* (KAUNHM201501, KAUNHM201502, KAUNHM201503 and KAUNHM201504). Tissues were preserved in absolute ethanol. DNA extraction, PCR amplification of 16S rRNA gene and sequencing protocols followed Padhye et al. (2014). Sequences were analyzed by the BLAST tool (Altschul et al. 1990). These sequences have been deposited in GenBank under the accession numbers KP826810 to KP826820 and KT334413. Additional 16S gene sequences were retrieved from the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/). GenBank accession numbers for the sequences used in the study are provided in Appendix A. Gene sequences were aligned separately using MUSCLE (Edgar 2004). Molecular phylogenetic analysis was performed using MEGA 6 (Tamura et al. 2013). Pairwise raw phylogenetic distances were calculated in MEGA 6 (Tamura et al. 2013). The best fit model for nucleotide substitution was selected from 24 models using MEGA 6 (Tamura et al. 2013) based on the minimum Bayesian Information Criterion (BIC) value (Schwarz 1978; Nei & Kumar 2000). The best fit nucleotide substitution model was used for testing the phylogenetic hypothesis using maximum likelihood method. This analysis was not carried out to thoroughly resolve the deep phylogeny of the genus but to assign individuals to genetically homogenous clusters. Reliability of the phylogenetic tree was estimated using bootstrap values from 1000 replicates. Phylogenetic tree was edited in FigTree v1.4.2 (Rambaut 2009).

Comparative material

*Hydrophylax malabaricus* (n = 10): Lectotype, MNHN 4440, 67.1 mm SVL, female, Malabar, coll. Dussumier; Paralectotype, 1 ex., MNHN 1899.3451, 63.3 mm SVL, female, Malabar, coll. Dussumier; Paralectotype, 2 exs., MNHN 0771, 59.4 mm SVL, and MNHN 1989.3452, 56.2 mm SVL, females, Côte de Malabar, Inde (=Coast of Malabar, India); 2 exs., MNHN 4439, 52.2 mm SVL, and MNHN 1989.3448, 40.3 mm SVL, Juvenile, Bengale, Inde (=Bengal, India); 4 exs., KAUNHM201501-04, 35.5–38.1 mm SVL, Kerala Agricultural University, Main Campus (10.563°N & 76.275°E, 38m), Thrissur, Kerala, India, coll. P.O. Nameer on 22 January 2015.

*Hydrophylax leptoglossa* (n = 9): 3 exs., syntypes, MCZ A-1588, A-125024 and A-125025, Myanmar: Yangon, Rangoon, Burmah Rangoon, Burmah (16.783N, 96.167E), by W. Theobald (only photographs examined), 6 exs., MNHN 1893.458-463, Burma (only photographs examined). Additional data from Cope (1868), Bouleneger (1920), Lalremsanga et al. (2007) and Biju et al. (2014).

*Hydrophylax gracilis* (n = 1): 1 ex., UWZM 233029, 35.4 mm SVL, coll. Gravenhorst (only photographs examined). Additional data from Gravenhorst (1829), Bouleneger (1920) and Biju et al. (2014).

RESULTS

Phylogenetic analysis

For the 16S rRNA gene sequences, model test suggested Tamura (1992) nucleotide substitution model with invariant sites (T92 + I, BIC = 3284.19, lnL = -1282.61, I = 0.69) as the best nucleotide substitution model. *Hydrophylax bahuvistara* is a sister taxa to *H. malabaricus*, however it formed a distinct monophyletic group (Fig. 1). Raw genetic distance between *H. bahuvistara* and *H. malabaricus* was 4.0–4.5 %, which
is more than the mean threshold value of 2.6% for species delineation based on 16S rRNA gene sequences suggested by Biju et al. (2014). Raw genetic distance between *H. bahuvistara* and *H. gracilis* was 6.9–8.0%, while between *H. bahuvistara* and *H. leptoglossa* was 7.8–8.5%. Intraspecies genetic distance among *H. bahuvistara* samples (n = 18) collected from widely separated localities from Karnataka, Goa, Maharashtra and Madhya Pradesh ranged between 0.0 to 0.7% (Fig. 2).

**Morphometric analysis**

Size-adjusted morphometric data was not significantly different from multivariate normal (within group Doornik and Hansen omnibus $E_p = 33.21, P = 0.2281$). *Hydrophylax bahuvistara* type material, *H. malabaricus* types and topotypes, and *H. gracilis*, formed significantly different clusters (MANOVA, Pillai’s trace = 1.83, $F_{28,54} = 20.68, P < 0.0001$). *Hydrophylax bahuvistara* and *H. malabaricus* formed significantly different clusters (Fisher’s distance = 17.47, $P < 0.0001$) in DA (Fig. 3).

**Figure 1. Maximum likelihood tree based on 16S rRNA gene sequences using Tamura (1992) nucleotide substitution model with invariant sites ($T92 + I$, $BIC = 3284.19$, $lnL = -1282.61$, $I = 0.69$). *Hydrophylax bahuvistara* sp. nov. sequences generated in the present study are shown in red. Sequences in GenBank that should be attributed to *H. bahuvistara* sp. nov. are shown in blue. *Hydrophylax malabaricus* sequences of the topotypes are shown in green. Values along the nodes are percent bootstrap values for 1000 iterations. *Clinotarsus curtipes* is used as an outgroup.**
were mainly separated on the second axis of DA (Fig. 3) with characters such as HL, NS, UEW and HW having high factor loading in the direction of separation of *H. malabaricus*, indicating that these variables had higher values in *H. gracilis* than *H. bahuvistara*.

**Taxonomy**

*Hydrophylax bahuvistara* sp. nov.  
(Images 1–4)  
urn:lsid:zoobank.org:act:3CD3CD5B-FAC5-48AF-B256-E52281A75B1A

*Hylarana malabarica* haplogroup 1: Biju et al. (2014)

**Common name**

Wide-spread Fungoid Frog.

**Type material**

Holotype: BNHS 5921, female, 19.vi.1998, 80.1mm SVL, Tamhini, Mulshi (18.447°N & 73.431°E, 620m), Pune District, Maharashtra, India, by A.D. Padhye and N. Dahanukar.

Paratypes (n = 18): details in Table 1.

**Additional material**

Details of additional material studied is provided in Appendix B.

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**Figure 2.** Within species and between species raw genetic distances in *Hydrophylax bahuvistara* sp. nov. and *H. malabaricus*. Dashed line at 2.6% is the mean threshold value for species delineation following Biju et al. (2014).

**Figure 3.** Discriminant analysis of size-adjusted morphometric data. Values in parenthesis are percent variance explained by each discriminant axis. Pairwise Fishers distances (blue cells) and corresponding P values (red cells) are provided in the inset.
Hydrophylax bahuvistara sp. nov. can be separated from its congeners based on a combination of characters including HW/HL ratio in the range of 0.83–0.98, IUE/HL ratio in the range of 0.27–0.36, outline of snout in dorsal view truncated, finger and toe tips without lateroventral groove, foot moderately webbed with webbing formula I1¾-2II1-3III2-3½IV3½-2V, metatarsals of 4th and 5th toes closely set, outer metatarsal tubercle small, foot length 39.6–49.4 % of SVL, dorsal parts of shank without glandular folds and sparse horny spinules, and heels touch each other when the legs are folded at right angles to the body.
Description

Morphometric measurements are listed in Table 2. General body topology as in Image 1 and 2. Palm and foot structures as in Image 3. Coloration in life as per Image 4.

Description of holotype: Adult female BNHS 5921, Image 1 (all measurements in mm)

Medium sized (SVL 80.1), with moderately robust body; head length (HL 24.9) slightly longer than head width (HW 24.0); outline of snout in dorsal view truncated (Image 1a); snout length (SL 10.5) slightly longer than horizontal diameter of eye (EL 9.7); canthus rostralis angular, canthal region obtuse, loreal region concave; tympanum (TYD 6.7) distinct, rounded, almost ¾ of the eye diameter; interorbital distance (IUE 7.7) about 1½ times greater than width of upper eyelid (UEW 5.4). Inter orbital space flat.

Fore limbs: hand length (HAL 18.6) > forelimb (FAL 17.1); fingers long, thin, with flattened tips with rounded edges, without groove; subarticular tubercles prominent, oval, single; single inner palmer tubercle and double outer palmer tubercles present; supernumerary tubercles distinct; finger length formula II < I < IV < III (Image 3a).

Hind limbs: moderately long, tibio-tarsal articulation reaches middle of eye; thigh (TL 32.6), shank (SHL 32.8) and foot (FOL 33.1) subequal in length; toes long, thin, with flattened tips with rounded edges, without groove; webbing moderate, reaching between the 3rd and 4th subarticular tubercles on either side of toe IV; subarticular tubercles single, oval; supernumerary tubercles absent; dermal ridge on the outer side of the toe V present; tarsal fold absent; toe length formula 1 < 2 < 3 ≈ 5 < 4; inner metatarsal tubercle short, oval and distinct; outer metatarsal tubercle small, prominent

| Accession number | Sex | SVL (mm) | Locality | Latitude (°N) | Longitude (°E) | Altitude (m) | Collector | Date |
|------------------|-----|----------|----------|---------------|---------------|-------------|-----------|------|
| BNHS 5926        | M   | 68.8     | Chavani, Raigad, Maharashtra | 18.723       | 73.335        | 103         | Vivek Gour Broome | 8.vi.2002 |
| BNHS 5927        | M   | 64.6     | Paud, Pune, Maharashtra     | 18.531       | 73.558        | 582         | Sameer Hiremath   | 5.vii.2011 |
| BNHS 5922        | M   | 73.3     | Tamhini, Mulshi, Pune District, Maharashtra | 18.447       | 73.431        | 620         | A.D. Padhye, A. Jadhav | 25.vi.2008 |
| BNHS 5924        | F   | 57.1     | Dhamapur, Sindhudurg District, Maharashtra | 16.038       | 73.596        | 20          | A.D. Padhye, R. Pandit, A. Jadhav | 19.vi.2009 |
| BNHS 5925        | M   | 54.7     | Veineswar, Ratnagiri District, Maharashtra | 17.430       | 73.212        | 88          | R. Pandit, Ankur Padhye | 24.vi.2010 |
| BNHS 5923        | M   | 62.9     | Belane, Sindhudurg District, Maharashtra | 16.265       | 73.709        | 48          | A.D. Padhye, A. Jadhav, R. Pandit | 19.vii.2009 |
| WILD-15-AMP-523  | F   | 56.5     | Visapur, Pune, Maharashtra | 18.723       | 73.491        | 1027        | Nikhil Modak      | 21.iii.2013 |
| WILD-15-AMP-522  | M   | 58.0     | Varoshi near Mahabaleshwar, Satara, Maharashtra | 17.869       | 73.751        | 843         | Nikhil Modak, Siddharth Kulkarni | 21.viii.2014 |
| WILD-15-AMP-519  | M   | 70.3     | Tamhini, Mulshi, Pune, Maharashtra | 18.447       | 73.431        | 620         | A.D. Padhye, N. Dahanukar | 21.vi.2000 |
| WILD-15-AMP-520  | M   | 73.1     | Tamhini, Mulshi, Pune, Maharashtra | 18.447       | 73.431        | 620         | A.D. Padhye, N. Dahanukar | 19.vi.1998 |
| WILD-15-AMP-517  | M   | 63.4     | Amboli, Sindhudurg District, Maharashtra | 15.963       | 73.998        | 699         | A.D. Padhye, A. Jadhav, R. Pandit | 20.vii.2009 |
| WILD-15-AMP-518  | M   | 56.2     | Amboli, Sindhudurg District, Maharashtra | 15.963       | 73.998        | 699         | G. Salekar        | 12.vi.2003 |
| WILD-15-AMP-521  | M   | 64.9     | Kolvan, Pune District, Maharashtra | 18.583       | 73.533        | 610         | A. Zambre         | 25.vii.2009 |
| WILD-15-AMP-555  | M   | 33.7     | Wadala Tukum near Tadoba, Chandrapur District, Maharashtra | 20.300       | 79.262        | 224         | Abhijeet Bayani   | 22.xi.2013 |
| WILD-15-AMP-556  | M   | 36.0     | Wadala Tukum near Tadoba, Chandrapur District, Maharashtra | 20.300       | 79.262        | 224         | Abhijeet Bayani   | 22.xi.2013 |
| WILD-15-AMP-554  | M   | 68.8     | Ghatghar, Pune District, Maharashtra | 19.283       | 73.700        | 746         | A.D. Padhye, A. Jadhav | 28.vii.2009 |
| WILD-15-AMP-553  | F   | 39.7     | Siddhagad, Thane District, Maharashtra | 19.147       | 73.504        | 300         | N. Modak         | 24.xii.2012 |
| ZSI-WRC A/1543   | M   | 54.3     | Jamnghavi, Pune District, Maharashtra | 18.856       | 73.452        | 248         | A.D. Padhye      | 21.vi.2011 |
| AGCZRL-AMPHIBIA-23 | M  | 53.0     | Dhamapur, Sindhudurg District, Maharashtra | 16.038       | 73.596        | 20          | A.D. Padhye, A. Jadhav, R. Pandit | 19.vii.2009 |
Hydrophylax bahuvistara sp. nov. Padhye et al.

Skin texture: Head smooth, finely granular on the dorsal side of the body, with moderately developed conspicuous dorsolateral glandular folds. Mandibular margin having thick granulations. Rictal gland present. Gular skin smooth. Skin on venter smooth, with coarse granulations on the posterior-ventral surface of the thighs. Ventral skin in the trunk region smooth, coarsely granular on the posterio-ventral surface of the thighs.

Image 4. Hydrophylax bahuvistara sp. nov. in life from different localities. (a) Amboli, (b) Dhamapur, (c) Chiplun, (d) Tamhini, (e) Tadoba, and (f) Bondla Wildlife Sanctuary, Goa. Specimens not collected.
Hydrophylax bahuvistara

Description of a male paratype: Adult male BNHS 5926, Image 2 (all measurements in mm)

Medium sized (SVL 68.8), with slender body; head length (HL 21.1) slightly longer than head width (HW 18.4); outline of snout in dorsal view truncated (Image 2a); snout length (SL 10.1) slightly longer than horizontal diameter of eye (EL 8.5); canthus rostralis angular, canthal region obtuse, loreal region concave; tympanum (TYD 6.6) distinct, rounded, almost ¾ of the eye diameter; interorbital distance (IUE 4.0) slightly greater than ½ width of upper eyelid (UEW 4.0). Inter orbital space flat.

Fore limbs: head length (HAL 17.6) > forelimb (FAL 12.9); fingers long, thin, with flattened tips with rounded edges, without groove; subarticular tubercles prominent, oval, single; single inner palmer tubercle and double outer palmer tubercles present; supernumerary tubercles distinct; finger length formula II < I < IV < III.

Hind limbs: moderately long, tibio-tarsal articulation

Table 2. Morphometric data (mm) of Hydrophylax bahuvistara sp. nov. type material. Key: H - Holotype; P - Paratype; other character abbreviations as described in methods section.

| Accession number | Type | Gender | Locality     | SVL  | HW  | HL  | TYD | SL  | IUE | UEW | EN  | SN  | EL  | FAL  | HAL  | TL  | SHL  | FOL  |
|------------------|------|--------|--------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|-----|------|------|
| BNHS 5921*       | H    | F      | Tamhini      | 80.1 | 24.0| 24.9| 6.7 | 9.5 | 7.7 | 5.4 | 7.7 | 2.4 | 9.7 | 17.1 | 18.6 | 30.4 | 34.6 | 33.1 |
| BNHS 5924        | P    | F      | Dhamapur     | 57.1 | 17.7| 18.1| 5.1 | 9.0 | 5.2 | 4.3 | 5.1 | 3.5 | 7.2 | 12.9 | 14.5 | 22.6 | 26.0 | 23.8 |
| WILD-15-AMP-523  | P    | F      | Visapur      | 56.5 | 18.0| 20.1| 5.7 | 9.1 | 6.5 | 4.6 | 5.8 | 2.3 | 7.7 | 12.9 | 15.4 | 23.2 | 27.6 | 27.1 |
| WILD-15-AMP-554* | P    | F      | Siddhagad    | 39.7 | 11.7| 13.3| 3.4 | 5.6 | 3.8 | 2.5 | 4.0 | 2.1 | 4.5 | 8.8  | 10.5 | 18.1 | 18.5 | 17.3 |

* used for genetic analysis
reaches middle of eye; thigh (TL 27.3), shank (SHL 31.4) and foot (FOL 30.5) almost equal in length; toes long, thin, with flattened tips with rounded edges, without groove; webbing moderate, reaching between the 3rd and 4th subarticular tubercles on either side of toe IV; subarticular tubercles single, oval; supernumerary tubercles absent; dermal ridge on the outer side of the toe V present; tarsal fold absent; toe length formula 1 < 2 < 3 = 5 < 4; inner metatarsal tubercle short, oval and distinct; outer metatarsal tubercle small, prominent; heels touch each other when the legs are folded at right angles to the body.

Skin texture: Head smooth, finely granular on the dorsal side of the body, with moderately developed conspicuous dorsolateral glandular folds. Mandibular margin having thick granulations. Rictal gland present. Gular skin smooth. Skin on venter smooth, with coarse granulations on the posterior-ventral surface of the thighs. Ventral skin in the trunk region smooth, coarsely granular on the posterio-ventral surface of the thighs.

Coloration

In life: Dorsum with a wide band of pale orange color extending from tip of snout to vent, interspersed occasionally with black spots. Golden yellow dorsolateral fold, extending from tympanum to groins, separates bright orange dorsum from a dark brown dorsolateral region. Dorsolateral region of body bears creamy to golden yellow spots on dark brown background. Belly creamy white with light brown marbling. Fore limbs and hind limbs dark, with creamy white to yellow marbling. Lateral region of head dark brown; mandibular margin and rictal gland golden yellow in color. Throat yellowish, marbled with brown.

In alcohol preservation (Image 1): Dorsum color changes from pale orange to yellowish-brown. The color of other body regions remains same as in live specimens.

Etymology

The species is named bahuvistara (Sanskrit: ‘bahu’ = wide, ‘vistara’ = spread) owing to its wide distribution in peninsular India.

Variations

In life, dorsal coloration varies from pale orange to reddish-orange (Image 4), which changes from pale orange to yellowish-brown and reddish-orange to crimson in alcohol preservation. Ventral coloration varies from plain creamy white to yellow to dark brown or black. Creamy white or yellow marbling usually present on the dark belly, while pale brown marbling may or may not be present on creamy white or yellow belly. Male individuals usually attain more yellowish color during the breeding season. Morphometric variations for male and female types are provided in Table 1 with mean and standard deviation for all characters.

Sexual dimorphism

Males of the species have single internal vocal sac visible as loose skin on the throat in live specimens; dark, swollen patch on upper arm – humeral gland and a prominent, thick nuptial pad, which are absent in females.

Distribution

Type material of the species comes from a wide distribution in the Western Ghats of Maharashtra and eastern Maharashtra, however, based on genetic data available in Kurabayashi et al. (2005), Biju et al. (2014) and Hasan et al. (2014) and localities for additional material from this study and distributional data for Hydrophylax malabarica, the species is widespread in peninsular India distributed in Maharashtra, Karnataka, Goa and Madhya Pradesh (Table 3; Fig. 4).

Habitat, ecology and natural history

Hydrophylax bahuvistara sp. nov. is usually found near human habitation and in agricultural fields. It is also found on the forest floor and near ephemeral or permanent water bodies, but mainly during breeding season. The eggs are laid in shallow water in the paddy fields or on the banks of small ponds or lakes. Calling behavior of an adult male is shown in movie clip (Appendix C). Adults usually gather in large numbers at potential breeding habitats. A loud chorus of calling males is heard at such places (Appendix D). The loud chorus is audible form a distance of up to a kilometer on quiet nights. Occasionally, smaller groups of adult males are also seen calling from the periphery of temporary rain water pools.

Comparison

Diagnosis of the genus and a key to the species of Hydrophylax are provided in Box 1. Hydrophylax bahuvistara sp. nov. can be separated from H. malabarica based on higher HW/HL ratio (0.83–0.98 vs. 0.79–0.83), outline of snout in dorsal view truncated (vs. outline of snout in dorsal view suboval), foot moderately webbed (Fig. 5) with webbing formula I1½-2II1-¾II2-3½III1½-3IV3-1½V (vs. foot extensively webbed with webbing formula I1½-2II1-2III1½-3IV3-1½V),
metatarsals of 4th and 5th toes closely set (vs. widely separated), outer metatarsal tubercle small (vs. outer metatarsal tubercle large, round, prominent), heels touch each other when the legs are folded at right angles to the body (vs. heels strongly overlap each other).

*Hydrophylax bahuvistara* sp. nov. differs from *H. gracilis* by having smaller foot (FOL 39.6–49.4 % of SVL vs. FOL 53.1–62.8 % of SVL) and reduced webbing on foot (Fig. 5) with webbing formula I1½-2II1-3III2-3IV3½-2V (vs. foot extensively webbed with webbing formula I1½-2II1-2II2-3IV3-1½V). Further, *H. bahuvistara* differs from *H. gracilis* by having dorsal parts of shank without glandular folds and sparse horny spinules (vs. with glandular folds and horny spinules) (Biju et al. 2014).

*Hydrophylax bahuvistara* sp. nov. differs from *H. leptoglossa* by the absence of lateroventral groove on finger and toe tips (vs. present) and eyes separated from each other with a greater distance with IUE/HL ratio of 0.27–0.36 (vs. eyes placed closer to each other with IUE/HL ratio of 0.16–0.26).

Figure 4. Distribution map for *Hydrophylax bahuvistara* sp. nov. and *H. malabaricus*. 
DISCUSSION

*Hydrophylax bahuvistara* sp. nov. is the fourth species in the recently resurrected genus *Hydrophylax*, which is distributed in Sri Lanka, India, Bangladesh, southern Myanmar, and western Thailand (Frost 2015). While, *H. gracilis* is restricted to Sri Lanka, *H. malabaricus* and *H. bahuvistara* are currently known only from India and *H. leptoglossa* is known from northeastern states of India, Bangladesh, Myanmar and Thailand. Within India, *H. malabaricus* is distributed in Kerala and Tamil Nadu, *H. bahuvistara* is distributed in Karnataka, Goa, Maharashtra and Madhya Pradesh, while *H. leptoglossa* is distributed in Assam, Mizoram, Tripura, and Meghalaya. Although *H. bahuvistara* is widely distributed in peninsular India, there was low genetic distance in the 16S rRNA gene sequence within the different populations of this species. Both genetically and morphologically *H. bahuvistara* is a sister taxon to *H. malabaricus*. Genetically, the two species form a monophyletic group distinct from other two species of *Hydrophylax*.

Biju et al. (2014) considered *H. bahuvistara* as “*Hylarana malabarica* Haplogroup 1” on the basis of genetic studies. They highlighted the close morphological resemblance of the haplogroup from *H. malabaricus* sensu stricto. However, our field observations, study of *H. malabaricus* type series and topotypes, and study of *H. bahuvistara* specimens from a wide range of its distribution suggests that the two species are morphologically distinct and can be delineated based on discrete characters.

All the six populations of *H. malabaricus* studied by Padhye et al. (2012) should now be attributed to the new species *H. bahuvistara* based on the genetic analysis presented in the current study. Padhye et al. (2012) showed that within the six populations of *H. bahuvistara* there are morphological as well as genetic variations in Randomly Amplified Polymorphic DNA markers. In the current study, however, we show that there is little genetic distance in the 16S rRNA gene among the populations of *H. bahuvistara*. Nevertheless, it is also true that the populations of *H. bahuvistara* are highly fragmented because of habitat fragmentation and therefore, the population variation reported by Padhye et al. (2012) could be due to recent fragmentation of populations of *H. bahuvistara*.

There is confusion regarding the type series of *Hydrophylax malabaricus*. Original description by Tschudi (1838) does not mention the number of specimens in the type series. However, Guibé (1950) mentioned six specimens as syntypes of *H. malabaricus* originating from Malabar, India and collected by Roux and Dussumier. While examining the type series of *H. malabaricus* we observed that four specimens MNHN
Table 3. Distribution locations for *Hydrophylax bahuvistara* sp. nov. and *H. malabaricus*.

| Species                  | Locality                                      | Latitude (⁰N) | Longitude (⁰E) | Altitude m | Reference                  |
|--------------------------|-----------------------------------------------|--------------|---------------|------------|----------------------------|
| *Hydrophylax bahuvistara* | Tamhini, Maharashtra                         | 18.447       | 73.431        | 620        | Current study              |
| *Hydrophylax bahuvistara* | Amboli, Maharashtra                          | 15.963       | 73.998        | 699        | Current study              |
| *Hydrophylax bahuvistara* | Dhamapur, Maharashtra                        | 16.038       | 73.596        | 20         | Current study              |
| *Hydrophylax bahuvistara* | Kolvan, Maharashtra                          | 18.583       | 73.533        | 610        | Current study              |
| *Hydrophylax bahuvistara* | Paud, Maharashtra                            | 18.531       | 73.558        | 582        | Current study              |
| *Hydrophylax bahuvistara* | Velneshwar, Maharashtra                      | 17.430       | 73.212        | 88         | Current study              |
| *Hydrophylax bahuvistara* | Belane, Maharashtra                          | 16.265       | 73.709        | 48         | Current study              |
| *Hydrophylax bahuvistara* | Jambhavli, Maharashtra                       | 18.856       | 73.452        | 748        | Current study              |
| *Hydrophylax bahuvistara* | Chavani, Maharashtra                         | 18.723       | 73.335        | 103        | Current study              |
| *Hydrophylax bahuvistara* | *Mahabaleshwar (Varoshi)*, Maharashtra       | 17.869       | 73.751        | 843        | Current study              |
| *Hydrophylax bahuvistara* | Visapur, Maharashtra                         | 18.723       | 73.491        | 1027       | Current study              |
| *Hydrophylax bahuvistara* | Wadala Tukum near Tadoba, Maharashtra        | 20.300       | 79.262        | 224        | Current study              |
| *Hydrophylax bahuvistara* | Ghatghar, Maharashtra                        | 19.283       | 73.700        | 746        | Current study              |
| *Hydrophylax bahuvistara* | Siddhagad, Maharashtra                       | 19.147       | 73.504        | 300        | Current study              |
| *Hydrophylax bahuvistara* | Bhondala Wildlife Sanctuary, Goa             | 15.448       | 74.096        | 102        | Current study              |
| *Hydrophylax bahuvistara* | Mollem National Park, Goa                    | 15.318       | 74.293        | 163        | Current study              |
| *Hydrophylax bahuvistara* | Chiplun, Maharashtra                         | 17.532       | 73.521        | 7          | Current study              |
| *Hydrophylax bahuvistara* | Bajipe, Karnataka                             | 12.950       | 74.893        | 13         | Kurabayashi et al. (2005), Hasan et al. (2014) |
| *Hydrophylax bahuvistara* | Amarkantak, Madhya Pradesh                   | 22.682       | 81.753        | 1034       | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Goa                                           | 15.068       | 74.181        | 193        | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Kanheri Caves, Maharashtra                   | 19.208       | 72.906        | 180        | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Bhimashankar, Maharashtra                    | 19.068       | 73.605        | 972        | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Khandala, Maharashtra                        | 18.760       | 73.374        | 541        | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Matheran, Maharashtra                        | 18.990       | 73.269        | 840        | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Phansad, Maharashtra                         | 18.455       | 72.926        | 221        | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Ratnagiri, Maharashtra                       | 16.983       | 73.300        | 9          | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Koyna Nagar, Maharashtra                     | 17.400       | 73.760        | 746        | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Tansa, Maharashtra                           | 19.620       | 73.262        | 170        | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Tungareshwar, Maharashtra                    | 19.409       | 72.894        | 77         | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Amboli, Maharashtra                          | 15.969       | 73.988        | 733        | Biju et al. (2014)         |
| *Hydrophylax bahuvistara* | Kachigebalii, Karnataka                      | 13.984       | 75.110        | 640        | Biju et al. (2014)         |
| *Hydrophylax malabaricus* | Thrisure, Kerala                             | 10.563       | 76.275        | 38         | Current study              |
| *Hydrophylax malabaricus* | Manalur, Palni Hills, Tamil Nadu             | 10.299       | 77.728        | 1018       | Biju et al. (2014)         |
| *Hydrophylax malabaricus* | Kannanore (= Kannur), Kerala                 | 11.875       | 75.370        | 21         | Biju et al. (2014)         |
| *Hydrophylax malabaricus* | Tellicherry (= Thalassery), Kerala           | 11.752       | 75.492        | 12         | Biju et al. (2014)         |
| *Hydrophylax malabaricus* | Ednad, Kasaragod District, Kerala            | 10.140       | 76.395        | 16         | Biju et al. (2014)         |
| *Hydrophylax malabaricus* | Meladoor, Kerala                             | 10.317       | 76.350        | 150        | Biju et al. (2014)         |
| *Hydrophylax malabaricus* | Mannuthy, Kerala                             | 10.524       | 76.294        | 40         | Biju et al. (2014)         |
| *Hydrophylax malabaricus* | Pullurampara, Kerala                         | 11.405       | 76.038        | 55         | Biju et al. (2014)         |
| *Hydrophylax malabaricus* | Nilambur, Kerala                             | 11.272       | 76.224        | 40         | Biju et al. (2014)         |
| *Hydrophylax malabaricus* | Mananthavady (= Mānantoddy), Kerala          | 11.807       | 76.010        | 766        | Biju et al. (2014)         |

* Identity ascertained using molecular data; # for this museum specimen Biju et al. (2014) mention the coordinates 9.859⁰N, 78.216 ⁰E, 118m elevation which are wrong.
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4440, MNHN 1989.3451, MNHN 0771 and MNHN 1989.3452 (Image 5), collected by Dussumier, originated from Malabar and two of the syntypes MNHN 4439 and MNHN 1989.3448 (Image 6) are labeled as originating from Bangale, Inde (= Bengal, India) on the specimen bottle. As there is a contradiction in the information provided in Guibé (1950) and the information available on the specimen bottle for the later two specimens, we have not considered them as syntypes of H. malabaricus and they are not included in the morphometric analysis.

Biju et al. (2014) designated MNHN 4440 as lectotype of H. malabarica. As a result other three specimens originating from Malabar, namely MNHN 0771, MNHN 1989.3451 and MNHN 1989.3452, are paralectotypes of H. malabaricus.

Six specimens MNHN 1893.458-463 from Burma (Myanmar) in the Muséum national d’Histoire naturelle, France, have two labels: “Rana malabarica” and “Rana granulosa”. These specimens have a close similarity with the syntypes of Hydrophylax leptoglossa (MCZ A-1588, A-125024 and A-125025) and therefore they should be identified as H. leptoglossa.

Currently, H. malabaricus is listed as a Least Concern species in IUCN Red List of Threatened Species, owing to its wide distribution in the peninsular India (Biju et al. 2004). However, based on the current study, the populations north of Kerala belong to H. bahuvistara, which is a widely distributed species in the peninsular India, while H. malabaricus appears to be restricted to the Western Ghats of Kerala and Tamil Nadu. It is therefore essential to reassess the conservation status of H. malabaricus.

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## Appendix A. Location, voucher number and GenBank accession numbers for species used for molecular analysis.

| Species                      | Location                          | Voucher Accession number | Reference               |
|------------------------------|-----------------------------------|--------------------------|-------------------------|
| Hydrophylax bahuvistara sp. nov. | Tamhini, MH, India                | BNHS-5921                | KT281144 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Amboli, MH, India                 | WILD-15-AMP-517          | KP826814 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Dhamapur, MH, India               | AGCZRL Amphibia 23       | KP826811 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Kolvan, MH, India                 | WILD-15-AMP-521          | KP826813 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Velpheshwar, MH, India            | BNHS 5925                | KP826815 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Belane, MH, India                 | BNHS 5923                | KP826816 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Jambhavli, MH, India              | ZSI-WRC A/1543           | KP826810 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Mahabaleshwar (Varoshi), MH, India | WILD-15-AMP-522          | KP826812 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Wadala Tokum near Taloba, MH, India | WILD-15-AMP-555          | KP867063 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Ghagghar, MH, India               | WILD-15-AMP-554          | KP867061 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Siddhagadhm, MH, India            | WILD-15-AMP-553          | KP867062 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Bondla Wildlife Sanctuary         | WILD-13-AMP-011          | KT334413 (This study)    |
| Hydrophylax bahuvistara sp. nov. | Amarkantak, MP, India             | SDBDU 2011.596           | KM068967 (Biju et al. 2014) |
| Hydrophylax bahuvistara sp. nov. | Amboli, MH, India                 | BNHS 5880                | KM068968 (Biju et al. 2014) |
| Hydrophylax bahuvistara sp. nov. | Bhimashankar, MH, India           | SDBDU 2011.1100a         | KM068969 (Biju et al. 2014) |
| Hydrophylax bahuvistara sp. nov. | Kachigebailu, KA, India           | SDBDU 2011.31           | KM068970 (Biju et al. 2014) |
| Hydrophylax bahuvistara sp. nov. | Bajpe, KA, India                  | - specimen released -    | AB167936 (Kurabayashi et al. 2005) |
| Hydrophylax bahuvistara sp. nov. | Bajpe, KA, India                  | - specimen released -    | ABS30579 (Hasan et al. 2014) |
| Hydrophylax malabaricus       | Thrissur, KE, India               | KAUNHM201501            | KP826817 (This study)    |
| Hydrophylax malabaricus       | Thrissur, KE, India               | KAUNHM201502            | KP826818 (This study)    |
| Hydrophylax malabaricus       | Thrissur, KE, India               | KAUNHM201503            | KP826819 (This study)    |
| Hydrophylax malabaricus       | Thrissur, KE, India               | KAUNHM201504            | KP826820 (This study)    |
| Hydrophylax malabaricus       | Meladoor, KE, India               | BNHS 5879                | KM068966 (Biju et al. 2014) |
| Hydrophylax gracilis          | Hiyare, Sri Lanka                 | DZ 1164                  | KM068933 (Biju et al. 2014) |
| Hydrophylax gracilis          | Ganemulla, Sri Lanka              | DZ 1156                  | KM068934 (Biju et al. 2014) |
| Hydrophylax gracilis          | Karawaddana, Sri Lanka            | DZ 1107                  | KM068935 (Biju et al. 2014) |
| Hydrophylax gracilis          | Kotagala, Sri Lanka               | DZ 1080                  | KM068936 (Biju et al. 2014) |
| Hydrophylax gracilis          | Nachchaduwa, Sri Lanka            | DZ 1049                  | KM068937 (Biju et al. 2014) |
| Hydrophylax gracilis          | Nachchaduwa, Sri Lanka            | DZ 1050                  | KM068938 (Biju et al. 2014) |
| Hydrophylax gracilis          | Udawatta Kele, Sri Lanka          | DZ 1173                  | KM068939 (Biju et al. 2014) |
| Hydrophylax gracilis          | Belihuloya, Sri Lanka             | MNHN 2000.614           | AY014376 (Kosuch et al. 2001) |
| Hydrophylax leptoglossa       | Kyaukpyu District, Myanmar        | CAS239886                | KR264065 (Oliver et al. 2015) |
| Hydrophylax leptoglossa       | Mymensingh, Kewatkahi, Bangladesh | IABHU 1897              | A8530526 (Hasan et al. 2012) |
| Hydrophylax leptoglossa       | Mymensingh, BAU Campus, Bangladesh | IABHU F2243            | A8530527 (Hasan et al. 2012) |
| Hydrophylax leptoglossa       | Sylhet, Golappganj, Bangladesh    | IABHU 3784              | A8530528 (Hasan et al. 2012) |
| Clinotarsus curtipes          | Karnataka                         | SDBDU 2011.42           | KM069013 (Biju et al. 2014) |
Appendix B. Additional material examined.

*Hydrophylax bahuvistara* sp. nov. (n = 7): 1 ex., 08.x.2012, WILD-13-AMP-011, road kill, Bondla Wildlife Sanctuary, Goa (15.448°N & 74.096°E, 102m), by Keerthi Krutha; 1 ex., photographic record, specimen not collected, Amboli (15.963°N & 73.998°E, 699m), Sindhudurg District, Maharashtra, by Hemant Ogale (Image 4a); 1 ex., 19.vi.2009, photographic record, specimen not collected, Dhamapur (16.038°N & 73.596°E, 20m), Sindhudurg District, Maharashtra, by A. Jadhav (Image 4b); 1 ex., photographic record, specimen not collected, Chipun (17.532°N & 73.521°E, 7m), by Ram Mone (Image 4c); 1 ex., 19 vi. 1998, photographic record, specimen not collected, Tamhini, Mulshi (18.447°N & 73.431°E, 620m), Pune District, Maharashtra, India, by A.D. Padhye (Image 4d); 1 ex., photographic record, specimen not collected, Wadala Tukum near Tadoba (20.3°N & 79.262°E, 224m), Chandrapur District, Maharashtra, by Nikhil Dandekar (Image 4e); 1 ex., 10.x.2012, photographic record, specimen not collected, Moliem National park (15.318°N & 74.293°E, 163m), Goa, by Keerthi Krutha (Image 4f).

Appendix C. Movie of *Hydrophylax bahuvistara* sp. nov. calling behavior. Video clip recorded on 6 July 2011 at Tamhini (18.447°N & 73.431°E, 620m) by Sanjay Khatakur, using Nikon COOLPIX 54000.

Appendix D. Audio clip of *Hydrophylax bahuvistara* sp. nov. calling in chorus. Audio clip recorded on 19 July 2009 at Dhamapur lake (16.038°N & 73.596°E, 20m a.s.l.) by Anand Padhye, using Cannon S2 IS.

**Author Contribution:** ADP, AJ, NM, PON and ND collected and studied the specimens; ADP and ND diagnosed the species; AJ Performed morphometry; ADP and NM studied the type material; ND performed molecular and statistical analysis; ADP, AJ, NM, PON and ND wrote the manuscript.

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