



ISSN 0974-7907 (Online)  
ISSN 0974-7893 (Print)

## HYDROPHYLAX BAHUVISTARA, A NEW SPECIES OF FUNGOID FROG (AMPHIBIA: RANIDAE) FROM PENINSULAR INDIA

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### OPEN ACCESS



**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 4<sup>th</sup> and 5<sup>th</sup> 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.

**DOI:** <http://dx.doi.org/10.11609/JoTT.o4252.7744-60> | **ZooBank:** urn:lsid:zoobank.org:pub:394965C1-5F9A-4E43-8F54-C352C75FAEAE

**Editor:** Annemarie Ohler, Muséum national d'Histoire naturelle, Paris, France.

**Date of publication:** 26 September 2015 (online & print)

**Manuscript details:** Ms # o4252 | Received 08 March 2015 | Final received 13 August 2015 | Finally accepted 27 August 2015

**Citation:** Padhye, A.D., A. Jadhav, N. Modak, P.O. Nameer & N. Dahanukar (2015). *Hydrophylax bahuvistara*, a new species of fungoid frog (Amphibia: Ranidae) from peninsular India. *Journal of Threatened Taxa* 7(11): 7744–7760; <http://dx.doi.org/10.11609/JoTT.o4252.7744-60>

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**Funding:** This work was partially supported by BCU, Pune University to Anand Padhye [BCUD/OSD/104] and partially by DST-INSPIRE Research Grant [IFA12-LSBM-21] to Neelesh Dahanukar. Nikhil Modak is supported by DST-INSPIRE Student Fellowship [IF 120398]. Nikhil Modak was supported by Ernst Mayr Travel Grant, MCZ, Harvard University, US, for examination of specimens at NHM, London and MNHN, Paris. P.O. Nameer's amphibian studies are supported by the funding from the Kerala Agricultural University.

**Conflict of Interest:** The authors declare no competing interests. Funding sources had no role in study design, data collection, results interpretation and manuscript writing.

For **Author Contribution** and **Author Details** see end of this article.

**Acknowledgements:** We thank Head of the Zoology and Biodiversity Departments and Principal, MES' Abasaheb Garware College, Pune and Indian Institute of Science Education and Research, Pune, for providing infrastructural facilities. We are thankful to Dr. H.V. Ghate for helpful discussions and valuable suggestions. We are grateful to Dr. Asad Rahmani, Director; Dr. Deepak Apte, COO; Rahul Khot, incharge Natural History Collection; and Vithoba Hegde, senior field assistant, for their help during study of the museum specimens and registration of specimens at Bombay Natural History Society (BNHS), Mumbai. NM is indebted to Dr. Annemarie Ohler of Amphibians and Reptiles section, Muséum national d'Histoire naturelle (MNHN) for her help and guiding discussions about taxonomy. NM is also thankful to the staff at MNHN for their kind help. We are thankful to Joseph Martinez, Herpetology section, Museum of Comparative Zoology, Harvard University, for photographs of *Hydrophylax leptoglossa* syntypes. We are also thankful to Bartosz Borczyk, Natural History Museum, Wroclaw University, for helping ADP and NM with examination of a specimen of *Hydrophylax gracilis*. We also thank Dr. P.S. Bhatnagar, officer-in-charge, and Dr. Shrikant Jadhav, Zoological Survey of India, Western Regional Center (ZSI-WRC), Pune, for their help in registering specimens in ZSI-WRC. We are thankful to Dr. Sanjay Molur and Keerthi Krutha for helping in registration of specimens in the Wildlife Information Liaison Development (WILD), Coimbatore. We are also thankful to Abhijeet Bayani, Rohan Pandit, Ankur Padhye, Vivek Gaubrome, Sameer Hiremath, Sheetal Shelke, Siddharth Kulkarni, Sandesh Apte, Ajit More, Raju More, Mandar Paingankar, Gautam Salelkar, Ravindra Gavari, Vitthal Bhoje, Vinayak Khade, Amod Zambre, Vivek Vishwasrao, Sanjay Khatakar, Satish Pande, Rajgopal Patil, Keerthi Krutha and Anil Pujari for helping with field work. We are grateful to Keerthi Krutha, Hemant Ogle and Ram Mone for providing photographs of live specimens. Observations and collections from Goa by Keerthi Krutha were carried out under the Goa forest department permit number 1-566-WL&ET/12-13/1034, dated 27/07/2012 and 2/21/GEN/WL&ET(S)/2012-13/4, dated 08/08/12.

## 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, Chandrapur 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), Hayward University. Photographs of *Hydrophylax gracilis* were obtained from Natural History Museum (UWZM), Wroclaw University.

### Morphometry

Measurements were taken to the nearest 0.1mm 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) was 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, 67.1mm SVL, female, Malabar, coll. Dussumier; Paralectotype, 1 ex., MNHN 1989.3451, 63.3mm SVL, female, Malabar, coll. Dussumier; Paralectotype, 2 exs., MNHN 0771, 59.4mm SVL, and MNHN 1989.3452, 56.2mm SVL, females, Côte de Malabar, Inde (=Coast of Malabar, India); 2 exs., MNHN 4439, 52.2mm SVL, and MNHN 1989.3448, 40.3mm 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), Boulenger (1920), Lalremsanga et al. (2007) and Biju et al. (2014).

*Hydrophylax gracilis* (n = 1): 1 ex., UWZM 233029, 35.4mm SVL, coll. Gravenhorst (only photographs examined). Additional data from Gravenhorst (1829), Boulenger (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

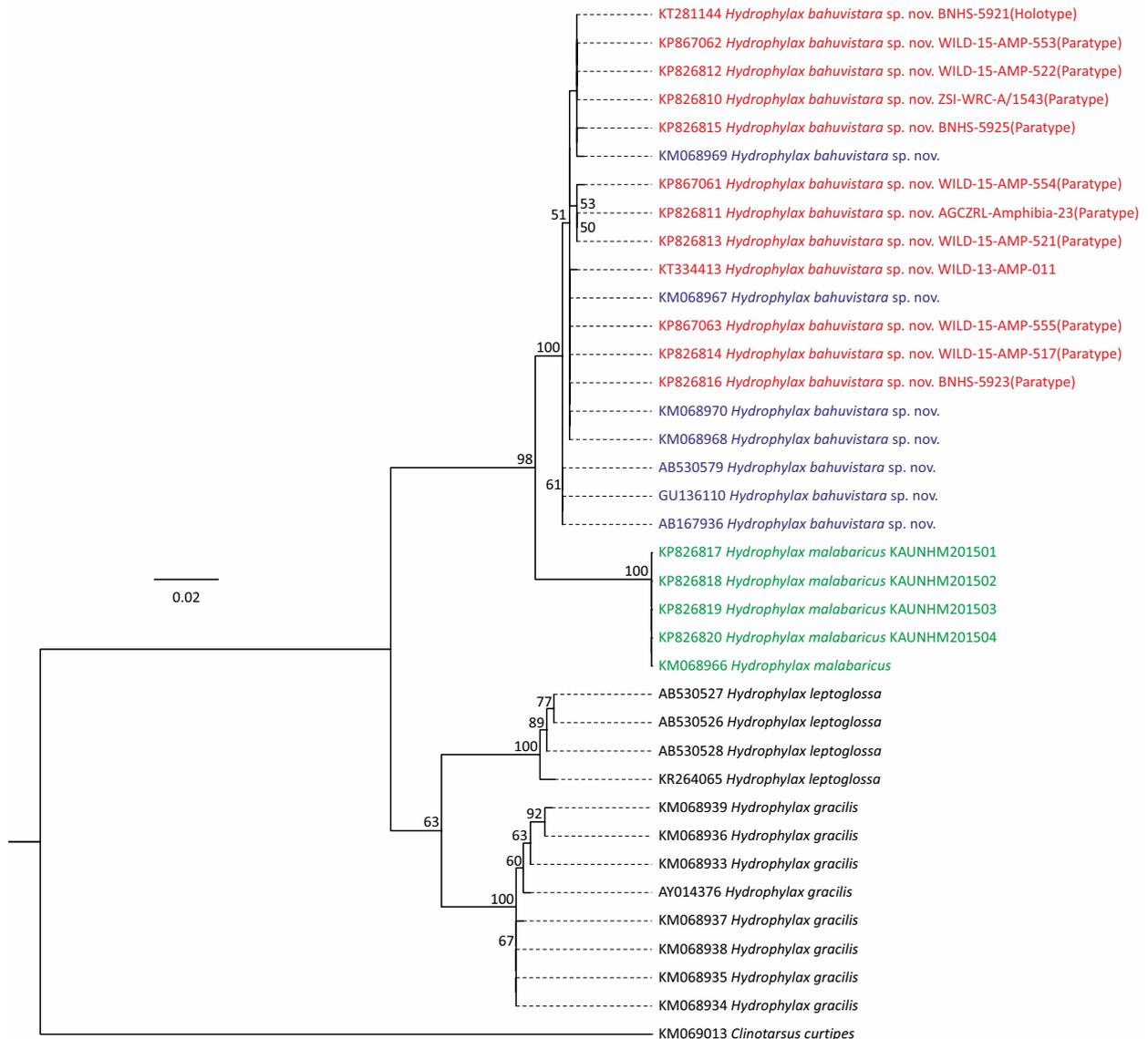
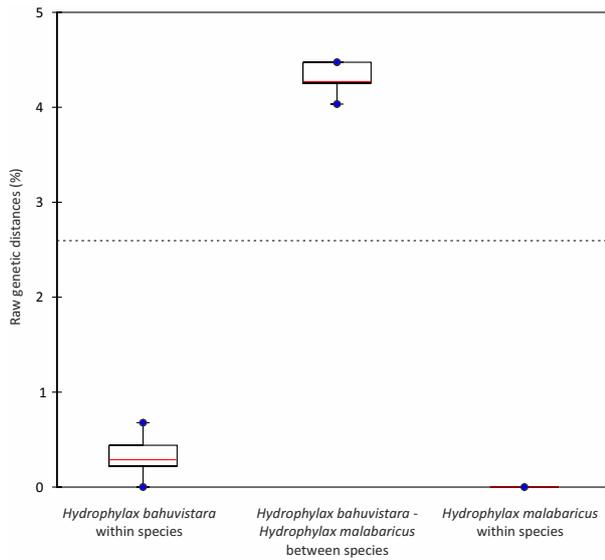


Figure 1. Maximum likelihood tree based on 16S rRNA gene sequences using Tamura (1992) nucleotide substitution model with invariant sites (T92 + I, BIC = 3284.19, lnL = -1282.61, I = 0.69). *Hydrophylax bahuvistara* sp. nov. sequences generated in the present study are shown in red. Sequences in GenBank 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.

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). *Hydrophylax bahuvistara* and *H. malabaricus*



**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).

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. malabaricus* than *H. bahuvistara*.

*Hydrophylax bahuvistara* and *H. gracilis* were separated on the first axis of DA (Fig. 3) with characters such as FOL, SHL and TL having high factor loading in the direction of separation of *H. gracilis*, 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.

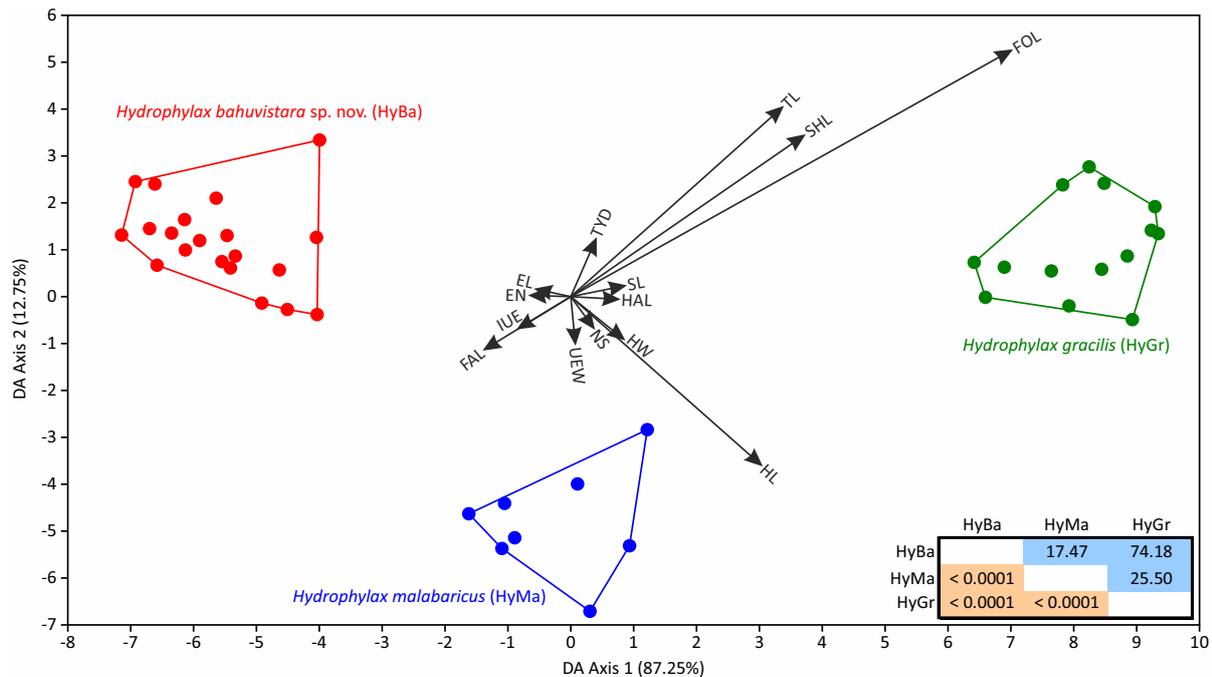
**Type material**

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.



**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.

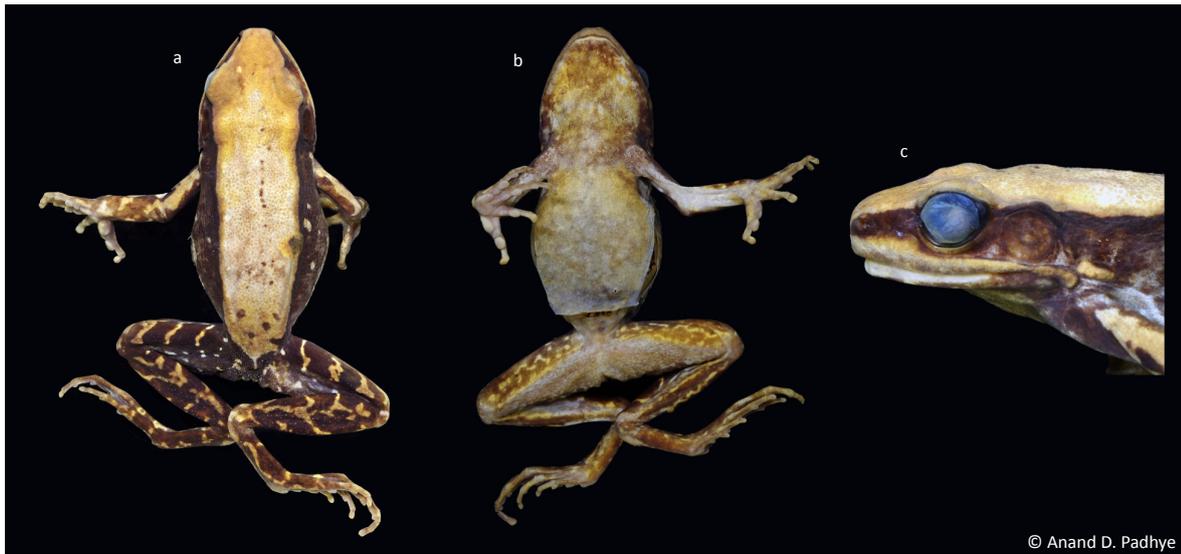


Image 1. *Hydrophylax bahuvistara* sp. nov. holotype BNHS 5921 (80.1mm SVL, female) in (a) dorsal, (b) ventral and (c) lateral view.



Image 2. *Hydrophylax bahuvistara* sp. nov. paratype BNHS 5927 (64.6 mm SVL, male) in (a) dorsal, (b) ventral and (c) lateral view.

### Diagnosis

*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  $1\frac{1}{4}-2\text{II}1-3\text{III}2-3\frac{1}{2}\text{IV}3\frac{1}{2}-2\text{V}$ , metatarsals of 4<sup>th</sup> and 5<sup>th</sup> 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.

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Image 3. Hand (a) and foot (b) of *Hydrophylax bahuvistara* sp. nov.

Table 1. Details of the paratypes of *Hydrophylax bahuvistara* sp. nov.

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.vii.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.vii.2009
BNHS 5925	M	54.7	Velneswar, 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. Salelkar	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.xi.2012
ZSI-WRC A/1543	M	54.3	Jambhavi, 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

## 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  $\frac{3}{4}$  of the eye diameter; interorbital distance (IUE 7.7) about  $1\frac{1}{2}$  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 3<sup>rd</sup> and 4<sup>th</sup> 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  $1 < 2 < 3 \approx 5 < 4$ ; inner metatarsal tubercle short, oval and distinct; outer metatarsal tubercle small, prominent



**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.

(Image 3b). 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 postero-ventral surface of the thighs.

**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-553*	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
			<i>Mean</i>	58.4	17.9	19.1	5.2	8.3	5.8	4.2	5.7	2.6	7.3	12.9	14.8	23.6	26.7	25.3
			<i>sd</i>	16.6	5.0	4.8	1.4	1.8	1.7	1.2	1.6	0.6	2.1	3.4	3.3	5.1	6.6	6.6
BNHS 5926	P	M	Chavani	68.8	18.4	21.1	6.6	10.1	7.6	4.0	7.0	3.5	8.5	12.9	17.6	27.3	31.4	30.5
BNHS 5925*	P	M	Velneshwar	54.7	16.0	17.5	6.0	9.2	4.9	3.7	5.7	2.9	6.1	10.4	14.6	23.8	25.1	25.1
BNHS 5923*	P	M	Belane	62.9	17.5	18.8	6.6	9.5	5.9	3.9	6.1	3.4	6.8	13.3	14.8	25.2	27.4	25.7
BNHS 5922	P	M	Tamhini	73.3	21.6	25.7	7.9	9.9	7.1	4.8	6.8	3.0	8.1	16.2	17.8	31.0	32.5	29.5
BNHS 5927	P	M	Paud	64.6	17.6	20.1	5.8	9.6	6.3	4.0	6.4	3.0	7.3	14.8	15.7	28.1	30.3	26.6
WILD-15-AMP-517*	P	M	Amboli	63.4	17.1	18.7	5.8	8.7	6.5	3.9	6.0	3.2	6.8	12.1	17.7	25.8	28.7	26.4
WILD-15-AMP-518	P	M	Amboli	56.2	15.5	17.1	5.8	8.5	5.1	3.7	5.5	1.6	7.3	12.6	13.3	24.0	25.1	23.4
WILD-15-AMP-519	P	M	Tamhini	70.3	18.3	22.0	6.5	8.9	6.7	4.8	6.3	3.6	8.1	13.9	16.9	29.7	32.7	29.4
WILD-15-AMP-520	P	M	Tamhini	73.1	21.2	22.7	7.8	9.5	7.3	4.7	6.6	2.3	8.2	15.2	16.6	29.9	30.6	30.0
WILD-15-AMP-521*	P	M	Kolvan	64.9	17.2	19.8	5.7	8.5	5.4	3.3	6.4	2.2	7.6	14.5	16.6	28.6	30.0	27.8
WILD-15-AMP-522*	P	M	Mahabaleshwar (Varoshi)	58.0	18.1	18.5	5.6	9.1	5.5	4.6	6.0	3.3	7.5	12.8	14.9	27.0	28.1	25.3
WILD-15-AMP-554*	P	M	Ghatghar	68.8	19.7	22.1	6.9	9.5	6.8	4.8	7.3	4.0	8.4	14.2	15.8	30.0	31.2	30.6
WILD-15-AMP-555*	P	M	Wadala Tukum near Tadoba	33.7	10.4	11.9	3.3	5.5	3.5	2.5	4.1	2.0	5.0	8.5	9.2	14.0	17.1	16.4
WILD-15-AMP-556	P	M	Wadala Tukum near Tadoba	36.0	11.7	13.6	3.4	5.8	4.0	2.9	4.8	2.0	5.2	7.8	10.8	17.3	19.3	17.8
ZSI-WRC A/1543*	P	M	Jambhavi	54.3	16.2	16.5	5.8	8.2	5.2	3.2	4.7	2.2	6.6	10.9	12.4	22.3	23.3	24.1
AGCZRL Amphibia 23*	P	M	Dhamapur	53.0	15.1	16.7	5.7	8.2	5.7	3.0	5.3	2.3	6.8	10.7	13.4	19.7	25.1	21.0
			<i>Mean</i>	59.8	17.0	18.9	6.0	8.7	5.8	3.9	5.9	2.8	7.1	12.6	14.9	25.2	27.4	25.6
			<i>sd</i>	11.7	3.0	3.5	1.2	1.3	1.2	0.7	0.9	0.7	1.1	2.4	2.5	4.9	4.6	4.3

\* used for genetic analysis

**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  $\frac{3}{4}$  of the eye diameter;

interorbital distance (IUE 7.6) slightly greater than  $\frac{1}{2}$  width of upper eyelid (UEW 4.0). Inter orbital space flat.

Fore limbs: hand 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

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 3<sup>rd</sup> and 4<sup>th</sup> 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  $1 < 2 < 3 \approx 5 < 4$ ; 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 postero-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* Haplogroup 1 from Biju et al. (2014), 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 from 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  $I1\frac{1}{4}-2II1-3III2-3\frac{1}{2}IV3\frac{1}{2}-2V$  (vs. foot extensively webbed with webbing formula  $I1\frac{1}{2}-2II1-2\frac{3}{4}III1\frac{1}{2}-3IV3-1\frac{1}{2}V$ ),

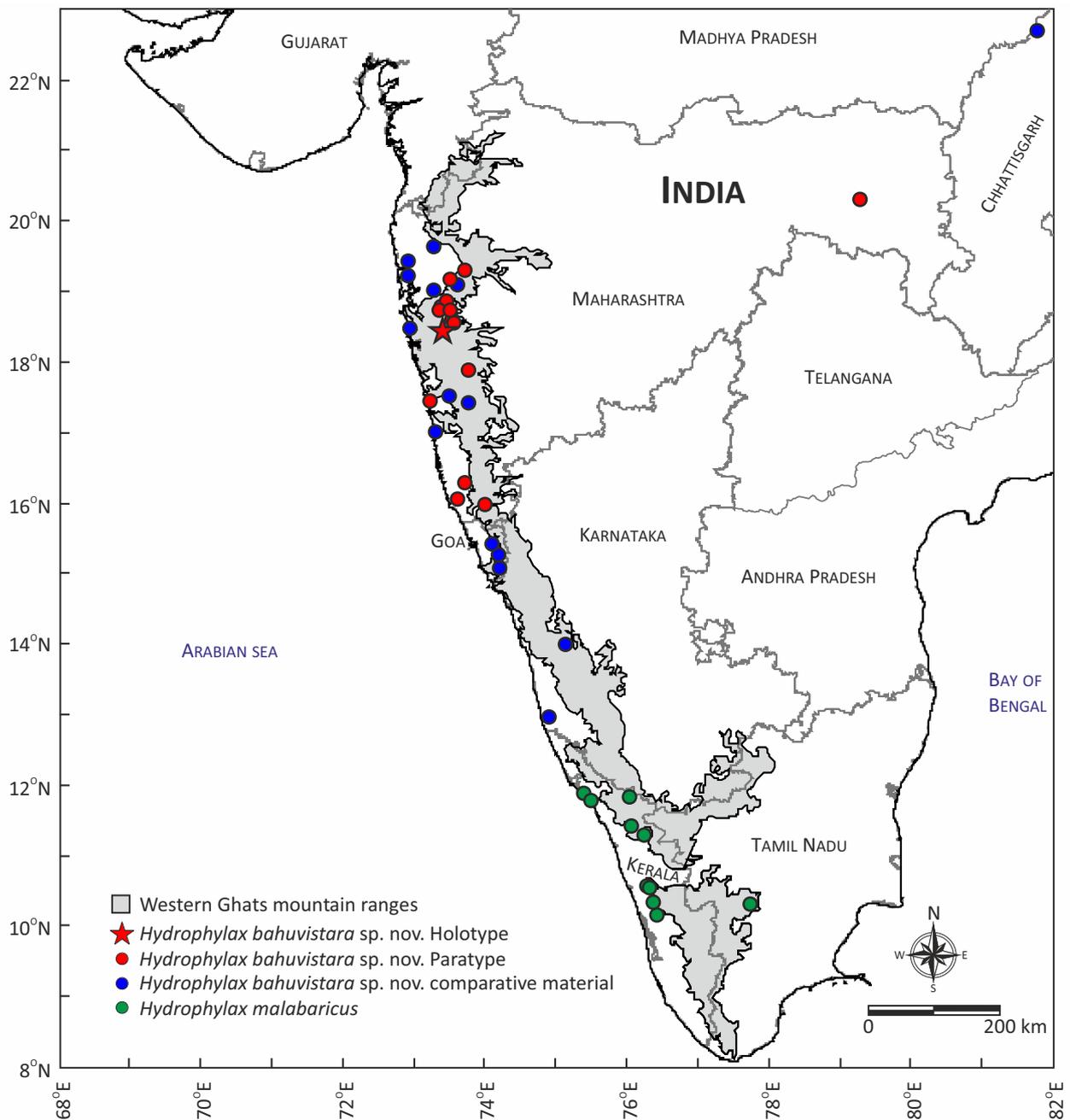


Figure 4. Distribution map for *Hydrophylax bahuvistara* sp. nov. and *H. malabaricus*.

metatarsals of 4<sup>th</sup> and 5<sup>th</sup> 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 I1¼-2II1-3III2-3½IV3½-2V (vs. foot extensively webbed with webbing formula

I1½-2II1-2III2-3IV3-1½V). 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).

Box 1. Key to the species of *Hydrophylax*

Robust body, dorsolateral folds moderately or well developed, rear of thighs with strong vermiculations, postocular mask present, large rectal gland, prominent humeral gland, circum-marginal grooves absent (except in *H. leptoglossa*), fourth toe webbing does not extend beyond the second subarticular tubercle on either side ..... *Hydrophylax*

1. Finger and toe tips with lateroventral groove ..... *leptoglossa*  
Finger and toe tips without lateroventral groove ..... 2
2. Foot length more than 50% of snout vent length, dorsal parts of shank with glandular folds and sparse horny spinules ..... *gracilis*  
Foot length less than or equal to 50% of snout vent length, dorsal parts of shank without glandular folds and sparse horny spinules .... 3
3. Foot extensively webbed with three phalanges of fourth toe free, heels strongly overlap each other when the legs are folded at right angles to the body ..... *malabaricus*  
Foot with reduced webbing with three and a half phalanges of fourth toe free, heels touch each other when the legs are folded at right angles to the body ..... *bahuvistara* sp. nov.

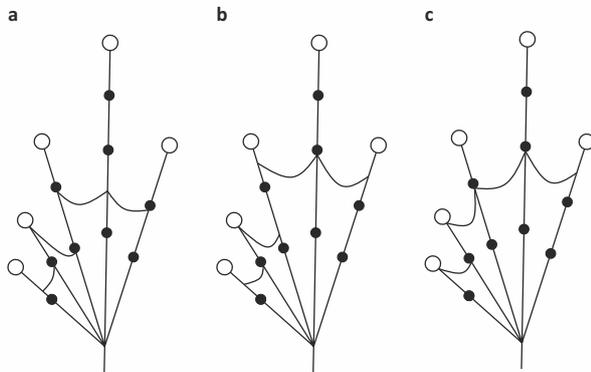


Figure 5. Illustration of extent of webbing in (a) *Hydrophylax bahuvistara* sp. nov., (b) *H. malabaricus* and (c) *H. gracilis*.

## 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 taxa 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 (°N)	Longitude (°E)	Altitude m	Reference
<i>Hydrophylax bahuvistara</i> *	Tamhini, Maharashtra	18.447	73.431	620	Current study
<i>Hydrophylax bahuvistara</i> *	Amboli, Maharashtra	15.963	73.998	699	Current study
<i>Hydrophylax bahuvistara</i> *	Dhmapur, Maharashtra	16.038	73.596	20	Current study
<i>Hydrophylax bahuvistara</i> *	Kolvan, Maharashtra	18.583	73.533	610	Current study
<i>Hydrophylax bahuvistara</i>	Paud, Maharashtra	18.531	73.558	582	Current study
<i>Hydrophylax bahuvistara</i> *	Velneswar, Maharashtra	17.430	73.212	88	Current study
<i>Hydrophylax bahuvistara</i> *	Belane, Maharashtra	16.265	73.709	48	Current study
<i>Hydrophylax bahuvistara</i> *	Jambhavli, Maharashtra	18.856	73.452	748	Current study
<i>Hydrophylax bahuvistara</i>	Chavani, Maharashtra	18.723	73.335	103	Current study
<i>Hydrophylax bahuvistara</i> *	Mahabaleshwar (Varoshi), Maharashtra	17.869	73.751	843	Current study
<i>Hydrophylax bahuvistara</i>	Visapur, Maharashtra	18.723	73.491	1027	Current study
<i>Hydrophylax bahuvistara</i> *	Wadala Tukum near Tadoba, Maharashtra	20.300	79.262	224	Current study
<i>Hydrophylax bahuvistara</i> *	Ghatghar, Maharashtra	19.283	73.700	746	Current study
<i>Hydrophylax bahuvistara</i> *	Siddhagad, Maharashtra	19.147	73.504	300	Current study
<i>Hydrophylax bahuvistara</i> *	Bhondala Wildlife Sanctuary, Goa	15.448	74.096	102	Current study
<i>Hydrophylax bahuvistara</i> *	Mollem National Park, Goa	15.318	74.293	163	Current study
<i>Hydrophylax bahuvistara</i> *	Chiplun, Maharashtra	17.532	73.521	7	Current study
<i>Hydrophylax bahuvistara</i> *	Bajipe, Karnataka	12.950	74.893	13	Kurabayashi et al. (2005), Hasan et al. (2014)
<i>Hydrophylax bahuvistara</i> *	Amarkhantak, Madhya Pradesh	22.682	81.753	1034	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i>	Goa	15.068	74.181	193	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i>	Kanheri Caves, Maharashtra	19.208	72.906	180	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i> *	Bhimashankar, Maharashtra	19.068	73.605	972	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i>	Khandala, Maharashtra	18.760	73.374	541	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i>	Matheran, Maharashtra	18.990	73.269	840	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i>	Phansad, Maharashtra	18.455	72.926	221	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i>	Ratnagiri, Maharashtra	16.983	73.300	9	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i>	Koyna Nagar, Maharashtra	17.400	73.760	746	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i>	Tansa, Maharashtra	19.620	73.262	170	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i>	Tungreshwar, Maharashtra	19.409	72.894	77	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i> *	Amboli, Maharashtra	15.969	73.988	733	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i> *	Kachigebailu, Karnataka	13.984	75.110	640	Biju et al. (2014)
<i>Hydrophylax malabaricus</i> *	Thrissure, Kerala	10.563	76.275	38	Current study
<i>Hydrophylax malabaricus</i> #	Manalur, Palni Hills, Tamil Nadu	10.299	77.728	1018	Biju et al. (2014)
<i>Hydrophylax malabaricus</i>	Cannanore (= Kannur), Kerala	11.875	75.370	21	Biju et al. (2014)
<i>Hydrophylax malabaricus</i>	Tellicherry (= Thalassery), Kerala	11.752	75.492	12	Biju et al. (2014)
<i>Hydrophylax malabaricus</i>	Edanad, Kasaragod District, Kerala	10.140	76.395	16	Biju et al. (2014)
<i>Hydrophylax malabaricus</i> *	Meladoor, Kerala	10.317	76.350	150	Biju et al. (2014)
<i>Hydrophylax malabaricus</i>	Mannuthy, Kerala	10.524	76.294	40	Biju et al. (2014)
<i>Hydrophylax malabaricus</i>	Pullurampara, Kerala	11.405	76.038	55	Biju et al. (2014)
<i>Hydrophylax malabaricus</i>	Nilambur, Kerala	11.272	76.224	40	Biju et al. (2014)
<i>Hydrophylax malabaricus</i>	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 9.859°N, 78,216 °E, 118m elevation which are wrong.

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.



**Image 5.** *Hydrophylax malabaricus* type series. Dorsal (a) and ventral (b) view of Lectotype MNHN 4440 (67.1mm SVL), dorsal view (c) of paralectotype MNHN 1989.3451 (63.3 mm SVL), dorsal view (d) of paralectotype MNHN 0771 (59.4 mm SVL) and dorsal view (e) of paralectotype MNHN 1989.3452 (56.2 mm SVL). Photo credit: Nikhil Modak.

Biju et al. (2014) designated MNHN 4440 as lectotype of *H. malabaricus*. 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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**Image 6.** Specimens of *Hydrophylax malabaricus*, with locality mentioned as Bangale, Inde (= Bengal, India). (a) Dorsal view & (b) ventral view of MNHN 4439 (52.2 mm SVL), and (c) dorsal view & (d) ventral view of MNHN 1989.3448 (40.3 mm SVL). Photo credit: Nikhil Modak.

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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
<i>Hydrophylax bahuvistara</i> sp. nov.	Tamhini, MH, India	BNHS-5921	KT281144	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Amboli, MH, India	WILD-15-AMP-517	KP826814	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Dhampur, MH, India	AGCZRL Amphibia 23	KP826811	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Kolvan, MH, India	WILD-15-AMP-521	KP826813	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Velneshwar, MH, India	BNHS 5925	KP826815	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Belane, MH, India	BNHS 5923	KP826816	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Jambhavi, MH, India	ZSI-WRC A/1543	KP826810	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Mahabaleshwar (Varoshi), MH, India	WILD-15-AMP-522	KP826812	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Wadala Tukum near Tadoba, MH, India	WILD-15-AMP-555	KP867063	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Ghatghar, MH, India	WILD-15-AMP-554	KP867061	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Siddhagadm, MH, India	WILD-15-AMP-553	KP867062	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Bondla Wildlife Sanctuary	WILD-13-AMP-011	KT334413	This study
<i>Hydrophylax bahuvistara</i> sp. nov.	Amarkantak, MP, India	SDBDU 2011.596	KM068967	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i> sp. nov.	Amboli, MH, India	BNHS 5880	KM068968	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i> sp. nov.	Bhimashankar, MH, India	SDBDU 2011.1100a	KM068969	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i> sp. nov.	Kachigebailu, KA, India	SDBDU 2011.31	KM068970	Biju et al. (2014)
<i>Hydrophylax bahuvistara</i> sp. nov.	Bajipe, KA, India	- specimen released -	AB167936	Kurabayashi et al. (2005)
<i>Hydrophylax bahuvistara</i> sp. nov.	Bajipe, KA, India	- specimen released -	AB530579	Hasan et al. (2014)
<i>Hydrophylax malabaricus</i>	Thrissur, KE, India	KAUNHM201501	KP826817	This study
<i>Hydrophylax malabaricus</i>	Thrissur, KE, India	KAUNHM201502	KP826818	This study
<i>Hydrophylax malabaricus</i>	Thrissur, KE, India	KAUNHM201503	KP826819	This study
<i>Hydrophylax malabaricus</i>	Thrissur, KE, India	KAUNHM201504	KP826820	This study
<i>Hydrophylax malabaricus</i>	Meladoor, KE, India	BNHS 5879	KM068966	Biju et al. (2014)
<i>Hydrophylax gracilis</i>	Hiyare, Sri Lanka	DZ 1164	KM068933	Biju et al. (2014)
<i>Hydrophylax gracilis</i>	Ganemulla, Sri Lanka	DZ 1156	KM068934	Biju et al. (2014)
<i>Hydrophylax gracilis</i>	Karawaddana, Sri Lanka	DZ 1107	KM068935	Biju et al. (2014)
<i>Hydrophylax gracilis</i>	Kotagala, Sri Lanka	DZ 1080	KM068936	Biju et al. (2014)
<i>Hydrophylax gracilis</i>	Nachchaduwa, Sri Lanka	DZ 1049	KM068937	Biju et al. (2014)
<i>Hydrophylax gracilis</i>	Nachchaduwa, Sri Lanka	DZ 1050	KM068938	Biju et al. (2014)
<i>Hydrophylax gracilis</i>	Udawatta Kele, Sri Lanka	DZ 1173	KM068939	Biju et al. (2014)
<i>Hydrophylax gracilis</i>	Belihuloya, Sri Lanka	MNHN 2000.614	AY014376	Kosuch et al. (2001)
<i>Hydrophylax leptoglossa</i>	Kyaukpyu District, Myanmar	CAS239886	KR264065	Oliver et al. (2015)
<i>Hydrophylax leptoglossa</i>	Mymensingh, Kewatkhali, Bangladesh	IABHU 3897	AB530526	Hasan et al. (2012)
<i>Hydrophylax leptoglossa</i>	Mymensingh, BAU Campus, Bangladesh	IABHU F2243	AB530527	Hasan et al. (2012)
<i>Hydrophylax leptoglossa</i>	Sylhet, Golapganj, Bangladesh	IABHU 3784	AB530528	Hasan et al. (2012)
<i>Clinotarsus curtipes</i>	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.vii.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, Chiplun (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, Mollem 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 Khatavkar, using Nikon COOLPIX S4000.**



**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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