VERTEBRATE ZOOLOGY

65 (3): 371 – 381
7.12.2015

SENCKENBERG

A taxonomic mystery for more than 180 years: the identity and systematic position of *Brachysaura minor* (Hardwicke & Gray, 1827)

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Accepted 19.x.2015.
Published online at www.senckenberg.de / vertebrate-zoology on 13.xi.2015.

Abstract

Brachysaura is a monotypic genus of agamid lizard found in the Indian subcontinent; the identity and systematic position of *B. minor* has been long debated, and it has at times been subsumed into *Agama, Charasia* and *Laudakia*, with some authors suggesting affinities to *Calotes*. We constructed nuclear and mitochondrial phylogenetic trees including *Brachysaura* and allied agamid genera to resolve its phylogenetic position. We also compared osteology and external morphology with the genera *Agama, Calotes* and *Laudakia*. Hemipenial morphology was compared with *Calotes* and some other agamids from South Asia. Both nuclear and mitochondrial phylogenies demonstrate that *Brachysaura* is nested within the widespread South and Southeast Asian genus *Calotes*, with which it also shares certain external morphological, osteological and hemipenial characters. Adaptations to ground dwelling in *Brachysaura minor* has resulted in unique modifications to its body plan, which is likely why generic allocation has been long confused. This study also highlights the need for an integrated systematic approach to resolve taxonomic ambiguity in Asian agamids.

Key words

Brachysaura, *Calotes*, genus, habit, morphology.

Introduction

Hardwicke & Gray (1827) described an interesting agamid with a very short tail from Chittagong, Bangladesh, as *Agama minor*. Their description was based on unpublished colour sketch by Hardwicke (Hardwicke NHM archives No. 82), which was in accordance with the figure (Smith 1935). Later Blyth (1856) described a new genus and species, *Brachysaura ornata*, based on specimen collected by Jerdon from Ságur, Central India. He considered this species as '*Calotes* with enormous head, short and thick body, the tail not exceeding the body in length, and the toes short and strong'. He also mentioned a weak nuchal crest, medial ridge with row of high-keeled scales and two detached tufts of sincipital spines. He distinguished this new genus based on the transverse arrangement of body scales, which are less obliquely oriented than in *Calotes*. Although Blyth's *B. ornata* appeared very similar to *Agama minor*, he did not refer to Hardwicke & Gray (1827). Later, Günther (1864) doubted the generic allocation of this species and noted that it was impossible to characterize the new genus *Brachysaura*, or fix its position in the family Agamidae based on the descriptions given by Blyth (1856). Subsequently Stoliczka (1872) reported this species from Kutch (then Kachha) as *B. ornata*. The specific

epithet *ornata* was followed by BOULENGER (1885), who also did not refer to HARDWICKE & GRAY (1827). Based on characters such as depressed body, very feeble dorsal crest, shoulder pits, a transverse throat fold, and a distinct tympanum, BOULENGER (1885) placed it in a new genus *Charasia*, along with *Psammophilus* spp. from the Indian subcontinent.

SMITH (1935) compared Hardwicke's painting (Hardwicke NHM archives No. 82) with the type of *Brachysaura ornata* and synonymized them, placing them in the genus *Agama*. Smith's (1935) generic allocation was based on the exposed tympanum and presence of callose preanal scales and therefore called it *Agama minor*. However he noted that the "the absence of callose preanal scales should not deter one from placing it under *Agama*" and gave an example of other species (*A. mutabulis*, *A. inermis* and *A. sinaita*) in the genus, which had reduced callose scales (SMITH 1935). These three species are presently placed in the genera *Trapelus* and *Pseudotrapelus* (UETZ & HOŠEK 2015). WERMUTH (1967) also followed SMITH (1935) and retained the name *Agama minor.* DAS (1994) erroneously placed this species in the genus *Laudakia,* mentioning that he followed nomenclature of MOODY (1980), though Moody had treated it as the monotypic genus *Brachysaura*. MANTHEY & SCHUSTER (1999) placed this species back into the genus *Brachysaura*. Subsequently, most authors have followed the genus name *Brachysaura* (DAS 2003, INGLE *et al*. 2012, KHAN 2006, KHAN & KUMAR 2010). Although MURTHY (2010) without any explanation moved this species to the genus *Laudakia*. Furthermore KHAN & KUMAR (2010) wrongly list *Acanthosaura minor* as one of the synonyms of *Brachysaura minor* although the former is a synonym of *Oriocalotes paulus* (SMITH 1935).

The taxonomic affinities of *Brachysaura minor* thus remain unclear. This species has been assigned to various genera of which *Charasia* is not valid any more. Most previous studies suggest that *Brachysaura minor* could be related to either *Calotes* (BLYTH 1856), *Agama* (SMITH 1935) or *Laudakia* (DAS 1994). We combined molecular and morphological data in an effort to resolve the chequered taxonomic history of *Brachysaura minor* and shed light on its evolutionary affinities.

Materials and methods

Sampling for this study is restricted to Gujarat state in India. Tissue sample were collected from two specimens, CESG 162 from Kutch and NCBS AQ035 from Chotila in Saurashtra. Remaining three specimens, CESG 466 is from Narayan Sarovar, BNHS 2307 is from Velavar and NCBS AQ036 is from Wardhwan. Genomic DNA was extracted from tissue samples that were stored in 99.9% ethanol and refrigerated at – 20°C. DNeasy® (Qiagen™) blood and tissue kit was used to extract DNA. Partial sequences of mitochondrial gene nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2; 1059 bp) and nuclear recombinant activating gene (RAG1; 940 bp) were PCR amplified and sequenced using published primers (MACEY *et al*. 1997, MACEY *et al*. 2000, GROTH & BARROWCLOUGH 1999). Additionally, ND2 and RAG1 sequences for agamids were downloaded from GenBank (Table S1). These sequences were aligned using ClustalW in Mega 5.1 (TAMURA *et al*. 2011). Uncorrected genetic distances between taxa were also calculated using MEGA 5.1. We used the GTR+I+G model for all phylogenetic analyses based on results from JModelTest 2.1.2 (DARRIBA *et al*. 2012). Trees were generated using both maximum likelihood (ML) and Bayesian approaches (BI). The ML tree was generated using the program RaXML GUI (SILVESTRO & MICHALAK 2012, STAMATAKIS *et al*. 2005) with bootstrap 500 reps in ML + rapid bootstrap settings. The program MrBayes 3.2 (RONQUIST *et al*. 2012) was used to generate the Bayesian tree with default prior settings. Markov chains were sampled every 1000 generations from a total of 1,000,000 generations, and the first 25% of the trees were discarded as "burn-in". Based on the findings by PYRON et al. (2013), *Brookesia brygooi* was used as an outgroup. To further test the relationship of *Brachysaura minor* with different genera the best ML tree was compared with alternative trees where *Brachysaura minor* was constrained to be sister to either *Laudakia* or *Agama*. These three tree topologies were compared using Shimodaira-Hasegawa (SH) test in PAUP 4.0b10 (SWOFFORD 2002)

Voucher Specimens were fixed in 4% formaldehyde for 24 hours and subsequently preserved in 70% ethanol for long-term storage. All morphometric measurements were taken using Mitutoyu™ digital vernier calipers (to the nearest 0.01mm). Most morphological characters were measured following ZUG *et al*. (2006), which are explained below. The following measurements were taken: snout-vent length (SVL, from tip of snout to anterior border of cloaca), head length (HL, distance from anterior edge of tympanum to tip of snout) head width (HW, distance from left to right outer edge of temporal at their widest), head depth (HH, at a point behind orbit), snout-eye length (SE, from snout tip to anterior border of orbit), eye to tympanum (EE, from posterior border of orbit to anterior border of tympanum), jaw length (JL, from rostrum to end of jaw), interorbital width (IO, transverse distance between anterodorsal corners of left and right orbits), naris to eye (NE, distance from the anterior edge of orbit to posterior edge of naris), snout width/internasal distance (IN, transverse distance between left and right nares), tympanum diameter (TD, greatest diameter of tympanum), orbit diameter (OD, distance between anterior and posterior margins of orbit), lower arm length (LAL, distance between elbow to upper side of forefoot), upper arm length (UAL, distance from anterior insertion of forelimb to elbow), finger lengths (F1, F2, F3, F4, F5), femur length (FL, length of femur from groin to knee), crus length (CL, length of crus (tibia) from knee to heel), hind foot length (HFL, distance from proximal end (heel) of hindfoot to distal most point of fourth toe

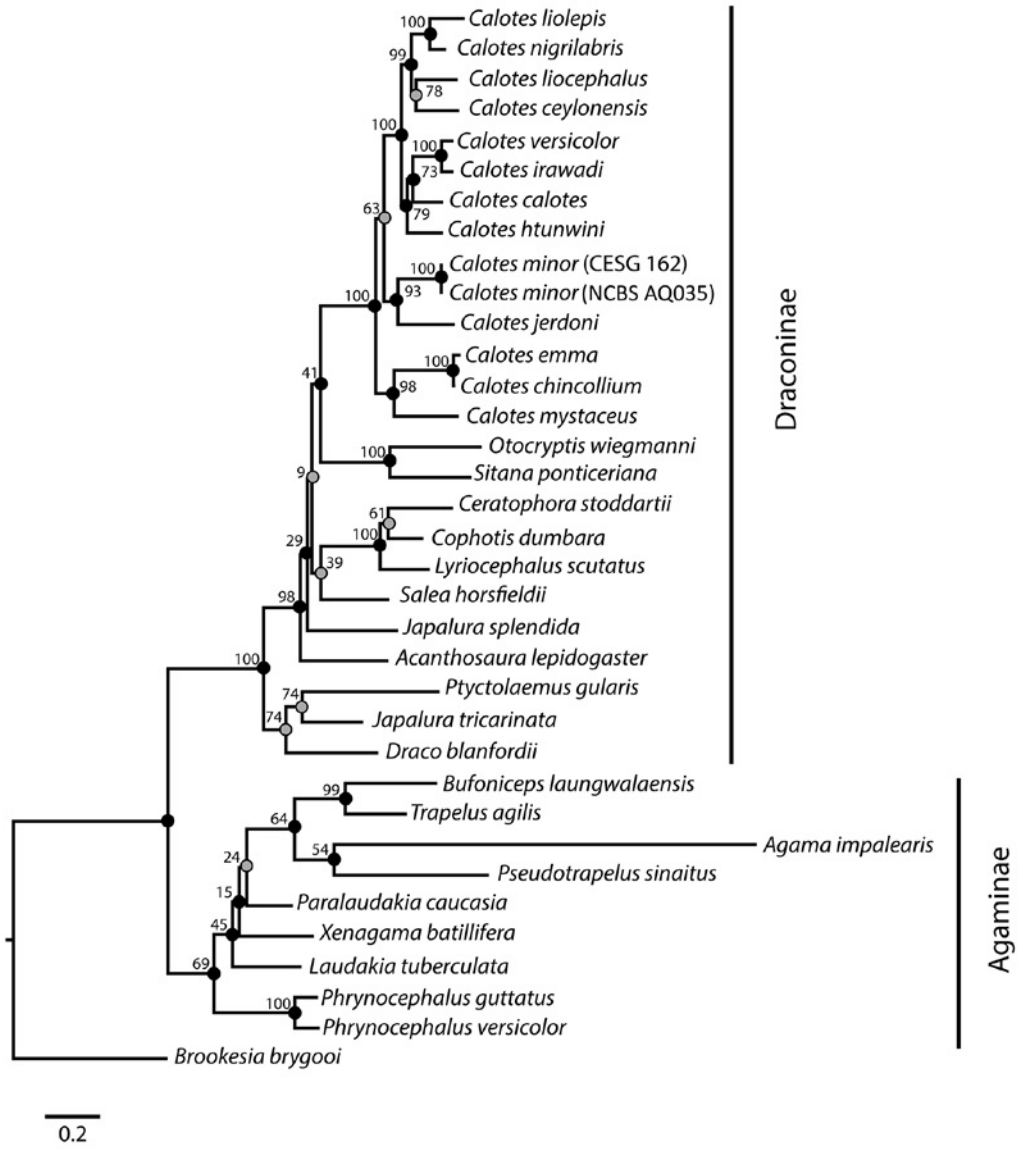

Fig. 1. Bayesian tree inferred from mtDNA data in MrBayes 3.2. The values assigned on the internodes indicate maximum likelihood bootstrap values and dark circles indicate posterior probability support above 95% and light circles indicate less than 95% probability.

excluding claw), toe lengths (T1, T2, T3, T4, T5) (e.g. T4 = Distance from juncture of 3rd and 4th digits to distal end of 4th digit on hindfoot excluding claw), torso length (TrL, from arm pit to groin), torso height (TrH, at mid-torso), torso width (TrW, at mid-torso), tail length (TL, from posterior border of cloacal opening to tip of tail), tail height (TH) and tail width (TW) at tail base. Following meristic characters were counted: mid-body scale rows (ABS, number of scale rows around trunk at mid-body), ventral scales (VEN, number of scales from below mental to cloacal opening for females, number of scales after the end of dewlap to cloacal opening for males), Number of subdigital lamellae (from 1st lamella at digits' cleft to most distal lamella, head scales (number of scales in transverse line between posteriormost left and right supraciliary scales), supralabials (SL, posterior end defined by posteriormost enlarged scales that touches infralabials at rear corner of mouth), Infralabial (IL, posterior end defined by posteriormost enlarged scales that touches with supralabials at rear corner of mouth).

Morphometric data of 19 different *Calotes* species were compiled from literature and museum collection (Amarsingae *et al*. 2014a, 2014b, Bahir & Maduwage 2005, Biswas 1975, Hallermann 2000, Hallerman & Böhme 2000, Hartmann *et al*. 2013, Ishwar & Das 1998, Vindum *et al*. 2003, Zhao & Li 1984, Zug *et al*. 2006) (Table S2). The original unpublished colour sketch of Hardwicke (Hardwicke NHM archives No. 82) was referred from the Natural History Museum Archives, London. We everted and prepared the hemipenis for two specimens of *Brachysaura minor* (NCBS AQ035and NCBS AQ036) while fixing, both collected during breeding season (July-August) (Ingle *et al*. 2012). Hemipenis drawings were done using a camera lucida attached to the microscope (Leica®™ M165C) and standard nomenclature (Dowling & Savage 1960) was used for the description. Hemipenial characters of *Brachysaura minor*

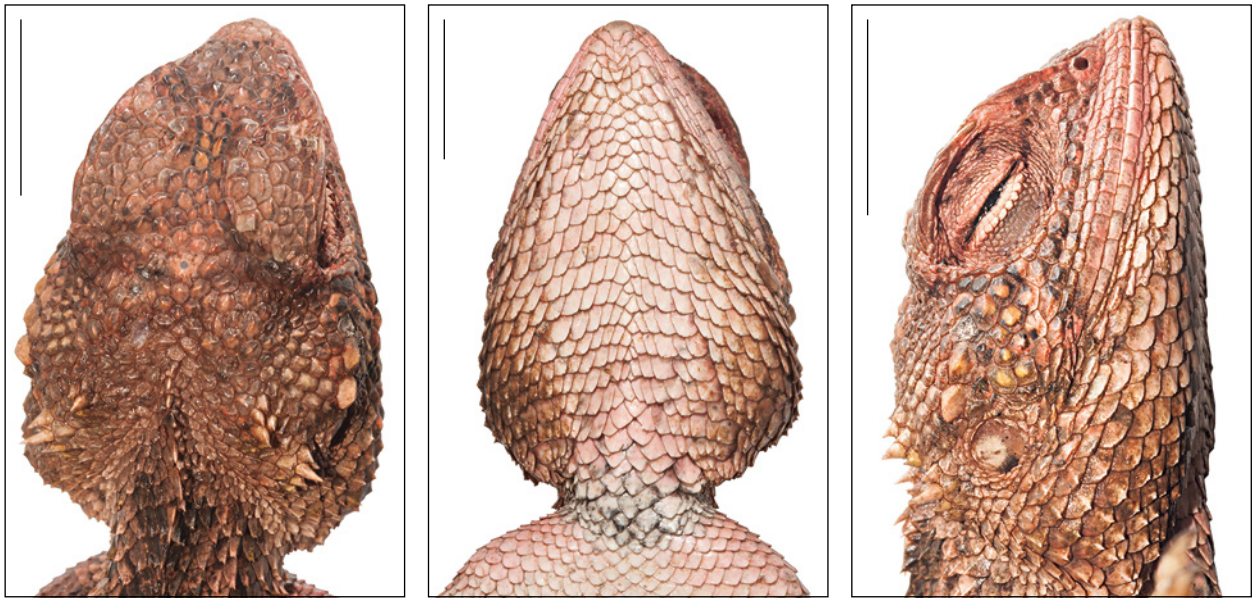

Fig. 2. Close up image of the head of *Brachysaura minor* (NCBS AQ036) collected from Wadhwan, Gujarat. Note the tuft of spines on the head and the row of enlarged scales/plate like between eye and tympanum. Scale bar: 10 mm.

were compared with other Draconine lizards (MADUWAGE *et al*. 2008). One adult female (SVL = 89.51 mm) of *Brachysaura minor* (CESG466) was clear stained following protocols from HANKEN & WASSERSUG (1981). Data on skeletal characters followed standard nomenclature (MOODY 1980, ROMER 1956). We compared select osteological characters specified in MOODY (1980) with data for *Calotes, Laudakia* and *Agama* (MOODY 1980).

Results

Phylogenetic relationship

In both ML and Bayesian trees built using mitochondrial sequences *Brachysaura minor* was nested within the wide spread Asian agamid genus *Calotes* with high bootstrap support and posterior probability (Fig. 1). Within the sampled *Calotes* clade, it is sister to *Calotes jerdoni*, an arboreal species found in Northeast India. Furthermore, the best tree, where *Brachysaura minor* is nested within *Calotes*, had a significantly higher likelihood score than the alternative trees (SH test, $P < 0.05$). The nuclear gene tree also had strong bootstrap support for *Calotes minor* being sister to *Calotes calotes* and not with *Laudakia* or *Agama* (Fig. S1).

Morphology

Majority of the description of *Brachysaura minor* based on specimens used in this and previous studies (BLYTH 1856, COCKBURN 1882, KHAN & KUMAR 2010, INGLE *et al*. 2012) are consistent with the type description of HARDWICKE & GRAY (1827). A detailed comparison of morphological characters of *Brachysaura minor* based on observations from current study and information available in earlier literature is made and provided here. Around the body scales 50–55 (current study) and 48–60 (KHAN & KUMAR 2010); 54–57 (INGLE *et al*. 2012); 48–50 (SMITH 1935). Ventral scales 71–85 (current study), no data available from earlier literature, the type sketch by Hardwicke (Hardwicke NHM archives No. 82) shows 68 ventrals. Supralabial and infralabials between 13–14 (current study, n = 4) and 10–15 (KHAN & KUMAR 2010, 'n' not provided); 12–14 (INGLE *et al*. 2012, n = 6); 11–15 (SMITH 1935, 'n' not provided). The following characters, although not mentioned in other literature but are consistent with the type description: two tufts of spines over the tympanum (Fig. 2), broad scales on the dorsum and a relatively short SVL to tail length ratio (Fig. 3).

To avoid the ambiguity there is a need to provide a revised morphological description for *Brachysaura minor*, based on published literature and the present study which is given as below. scales around the body 48–60, supralabial 10–15 and infralabials 10–15; dorsal and lateral scales large, moderately keeled, and larger than ventrals (Fig. 3); subdigital lamallae under the fourth toe 16–18, and subdigital lamallae under the fifth toe 8–9; tufts of spine above tympanum 2–6 and tufts of spine on the occiput region 5–6. They have 9–10 scales on the dorsal eyelids; 5–7 scales on the snout between the nostrils and 12–14 scales on the head between the posterior most supraciliary scales.

Brachysaura minor is a medium sized agamid with stout body and relatively short tail (Fig. 2; Table 1). This species have the shortest tail compared to 19 out of the 25 known species of *Calotes* (Fig. 4A, Table 1). *Brachy-*

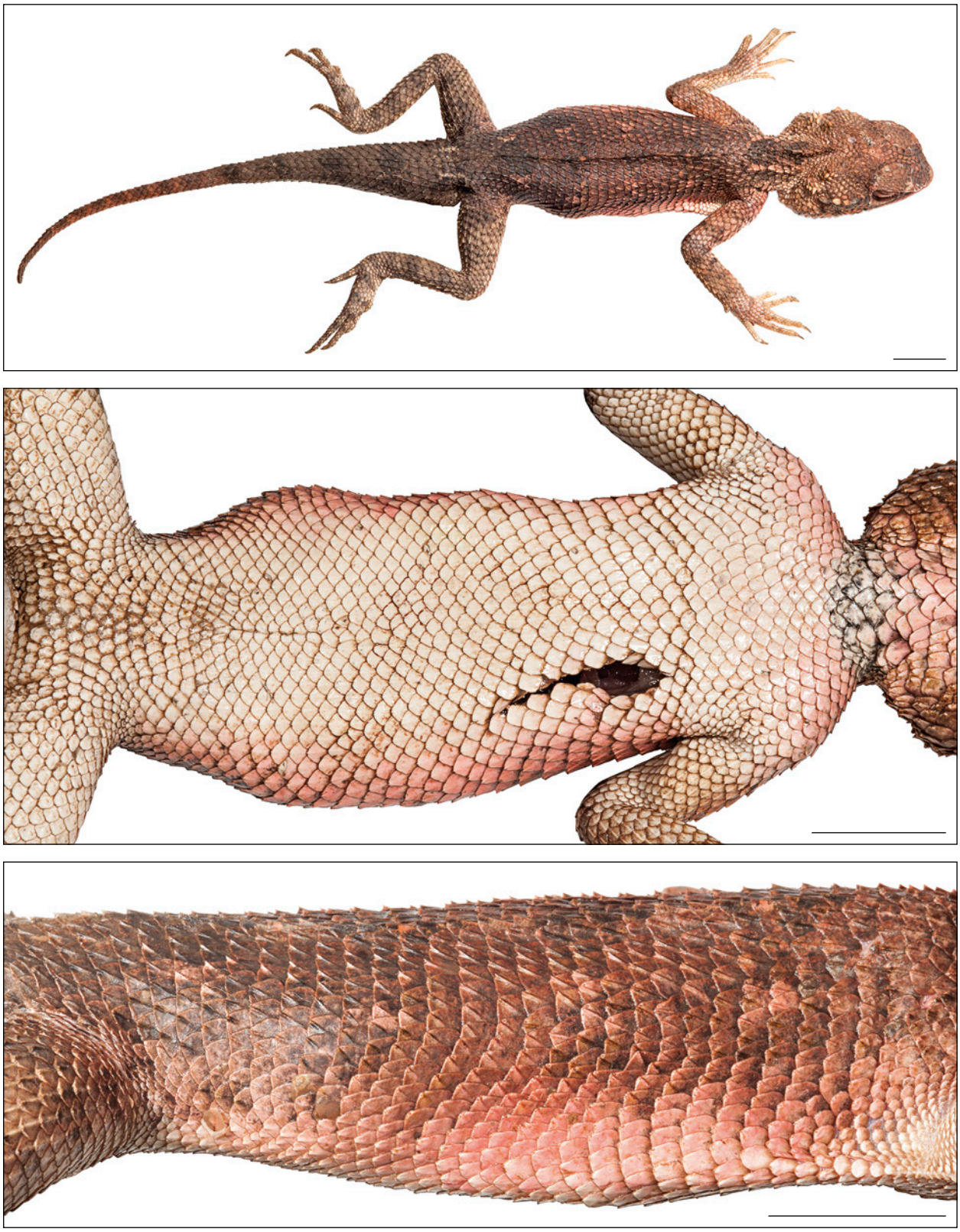

Fig. 3. Dorsal, ventral and lateral view of torso of *Brachysaura minor*, adult male (NCBS AQ036) collected from Wadhwan, Gujarat. Scale bar: 10 mm.

saura minor also have relatively short fifth toe compared to 14 other *Calotes* species (Fig. 4B, Table 1). It also have the widest head (HW/HL ratio = 0.79–0.82) compared to other *Calotes* (0.58–0.70 fide Hallerman & Böhme 2000). Like all the other members of *Calotes, B. minor* also have uniform dorsal body scale (Fig. 3). The tail length of *B. minor* is 88–99% of SVL and it is 190–300% in other *Calotes* excluding *Calotes calotes*, which has 320%. The hindlimb length of *B. minor* is 62–67 % of the SVL and for other *Calotes* it is 61–101%.

Table 1. Morphometric measurements of the four specimens used in this study.

	CESG 466	BNHS 2307	NCBS AQ035	NCBS AQ036
Sex	Female	Female	Male	Male
SVL	89.51	84.65	87.66	96.79
HL	24.34	23.82	24.31	25.65
HW	19.4	19.53	19.18	21
HH	14.69	15.43	15.34	16.09
EE	7.36	7.32	7.41	7.86
IO	9.65	8.95	10.36	10.28
JW	20.81	20.78	20	20.31
NE	3.89	4.19	4.37	4.42
SE	8.37	8.25	8.64	8.74
IN	5.59	5.25	5.76	6.61
TD	3.57	3.62	2.6	2.95
OD	7.95	6.94	7.77	7.9
LAL	16.04	15.91	16.38	17.61
UAL	14.17	14.93	15.5	15.76
F1	3.83	3.59	3.23	3.92
F2	6.34	5.46	5.51	6.08
F3	8.33	7.61	7.44	8.14
F4	7.01	7.6	7.28	6.97
F5	4.86	4.92	4.37	4.91
FL	17.47	18.13	17.37	20.16
CL	18.37	19.37	20.49	21.96
HFL	19.58	19.14	20.34	22.48
T1	3.5	3.52	2.89	4.46
T2	5.2	5.55	5.01	6.33
T3	7.13	8.03	7.53	8.71
T4	9.94	9.72	9.82	11.99
T5	5.17	5.48	5.71	6.66
TL	79.19	78.68	86.77	92.83
TW	8.84	8.12	10.27	10.5
TH	7.73	8.46	10.27	10.78
TrL	43.91	38.08	38.43	43.2
TrW	23.97	18.27	17.85	21.57
TrH	18.63	20.94	15.15	15.4

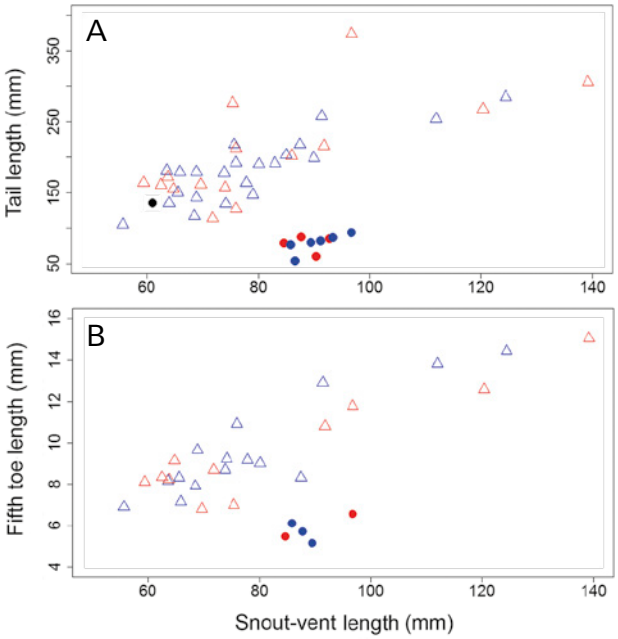

Fig. 4. Plot of body measurements of *Brachysaura minor* (blue circles (males), red circles (females)) compared to other known *Calotes* spp. (Blue triangles (males), red triangles (females)). Black circle *Calotes bhutanensis* sub-adult male. A) SVL versus tail length of 17 out of the 25 described species of *Calotes*. B) SVL versus fifth toe length of 9 out of the 25 described species of *Calotes*. The extremely short fifth toe and tail reflect its adaptation to terrestrial habits.

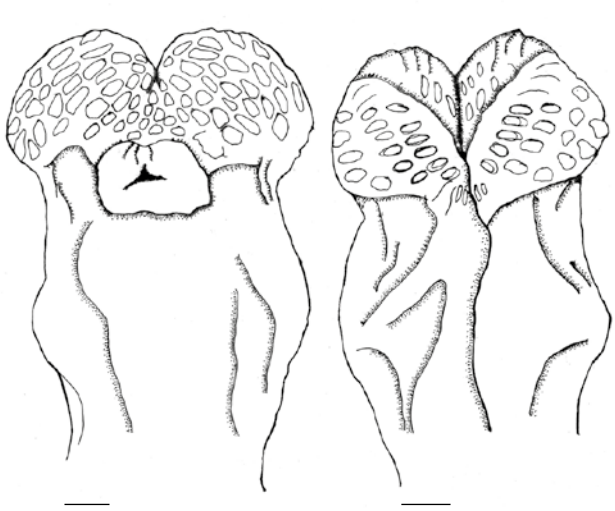

Fig. 5. Dorsal and ventral view of *Brachysaura minor* hemipenis shape and ornamentation. Scale bar: 1mm.

Hemipenial morphology

Hemipenis is single, clavate (divided less than half of its length), length of the entire organ greater than its width. Base naked, sulcus spermaticus single, proximal two third of sulcus spermaticus deep, dorsal half shallow. Lips of sulcus spermaticus smooth, widely open at the apex. A fleshy cardiod structure present at the base of ventral sulcus. Calyculate ornamentation present on each lobe. Thick walled smooth calyces forming deep oval pits. Apex capitate and are divided into four segments (Fig. 5). Hemipenis of *B. minor* matches with all the 14 characters reported from six other *Calotes* spp. from Sri Lanka and 13 out of the 14 characters with *Calotes ceylonensis* (Table 2).

Osteology

The skull of *Brachysaura minor* is subpentagonal in outline and raised in the parietal region and slopes steeply towards the nasal Fig. S2. The hyoid apparatus of *B. minor* is composed of six parts viz. 1. The entoglossal process; 2. The paired ceratohyals; 3. Ceratobranchials I; 4. Ceratobranchials II; 5. The paired epibranchials articulated posterior to the certobranchials I; 6. All the other five structures originate from a central body called the hyoid body. The Alzarin red stain suggest that most of the hyoid is bony, except the epibranchial I and II which is the tip of the respective ceratobranchials which are cartilaginous. Ceratobranchial II is more than half

Table 2. Hemipenial morphological characters used for comparison with *B. minor*. Character score "1" for yes and "0" for no. Numbers on top represents different species of *Calotes*: 1 (*C. calotes*), 2 (*C. nigrilabris*), 3 (*C. versicolor*), 4 (*C. ceylonensis*), 5 (*C. liolepis*), 6 (*C. liocephalus*), 7 (*C. desilvai*) and 8 (*Brachysaura minor*).

Character	1	2	3	4	5	6	7	8
Hemipenis divided for more than half its length	0	0	0	0	0	0	0	0
Flounces present	0	0	0	0	0	0	0	0
Apex of each lobe divided symmetrically both laterally and medially by sulcus	0	0	0	0	0	0	0	0
Sulcus spermaticus bifurcated	0	0	0	0	0	0	0	0
A fleshy cardioid structure present at the base of the ventral sulcus	1	1	1	1	1	1	1	1
Lateral and medial sulcus distinct throughout the length of each lobe	1	1	1	1	1	1	1	1
Length of entire organ greater than its width	1	1	1	1	1	1	1	1
Minute denticulation present on calyces	0	0	0	0	0	0	0	0
Sulcus traverses apex	0	0	0	0	0	0	0	0
Each lobe with more than 11 flounces	0	0	0	0	0	0	0	0
Ventral sulcus with transverse ridges	0	0	0	0	0	0	0	0
Transverse ridges present along more than half of length of the ventral sulcus	0	0	0	0	0	0	0	0
Calyces subequal along the entire length of the organ	0	0	0	0	0	0	0	0
Entire length of the lateral and medial sulcus with calyces	0	0	0	1	0	0	0	0

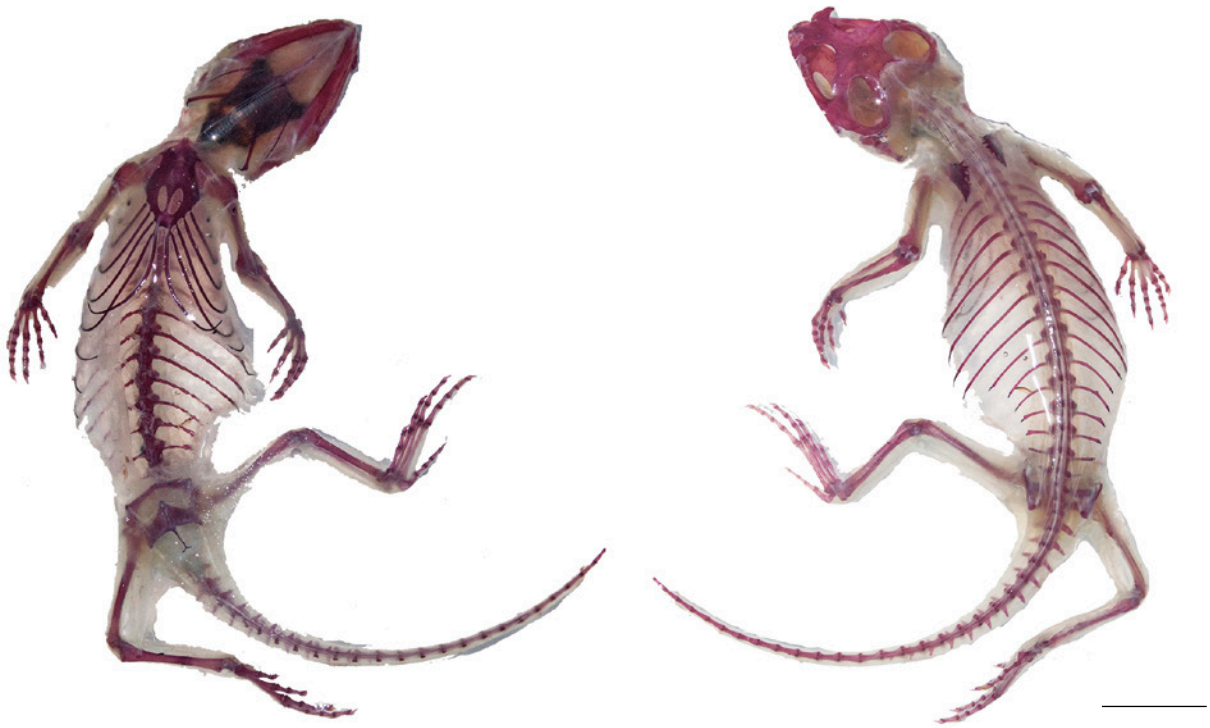

Fig. 6. Dorsal and ventral view of clear stained *Brachysaura minor* (CES G466). Note. The extreme tip of tail was broken and are not shown in this image. Scale bar = 15 mm.

the length of ceratobranchial I (Fig. 6). *Brachysaura minor* has 21 vertebrae excluding atlas and axis and 29 caudal vertebrae (Fig. 6). Sternum with a central foramen which is oval and elongated. Ten of the vertebrae had ribs and seven vertebrae has small ribs instead. The shoulder girdle is made of a broad clavicle; interclavicle long and rod like, suprascapula wedge shaped. The forelimbs is composed of a humerus with well-developed proximal and distal ends, ulna relatively thicker than radius. The phalange formula of the manus is 2 : 3 : 4 : 5 : 3. The hindlimb is formed by femur, tibia and fibula. The phalange formula on pes is 2 : 3 : 4 : 5 : 4. Thirteen out of sixteen characters examined in the skeleton of *B. minor* match with those of *Calotes* (Table 3). Only eight out of the sixteen characters compared matched with the genus *Laudakia* and only five out of the sixteen characters matched with the genus *Agama* (Table 3). Overall osteological characters of *B. minor* are more similar to *Calotes* and are different from *Laudakia* and *Agama* (Table 3).

Table 3. Comparisons of select osteological characters of *B. minor* and the three genera with which it was placed earlier. * In some species one or more phalange lost in 4th or 5th toe/finger.

Character	*Calotes minor*	*Calotes*	*Laudakia*	*Agama*
Meckelian groove remains on medial surface of dentaries	at the symphysis	at the symphysis	rotates to the ventral edge	rotates to the ventral edge
Size of jugal in the infraorbital region when viewed laterally	< half of maxilla	< half of maxilla	> maxilla	= maxilla
Medial edges of the palatal portion of the pterygoid	diverge posteriorly	remain parallel	diverge posteriorly	diverge posteriorly
Ceratobranchial (cb) II of hyoid apparatus	> half of cb I	> half of cb I	< half of cb I	< half of cb I
Tympanic membrane with surface and present external auditory meatus	absent	absent	present	present
Number of premaxillary acrodont teeth	3	3	2	1 or 2
Number of pleurodont teeth of premaxillae	15	11–13	12–15	8–10 or 5–7
Number of sternal ribs	2	2	2	3
Number of mesosternal ribs	2	2	2	1
Caudal vertebrae	29	46–65	35–45	35–45
Caudal vertebrae with transverse process	11	7–14	7–14	7–14
Number of trunk vertebrae excluding atlas and axis	21	21, 22 or 23	21, 22 or 23	21, 22 or 23
Width of sternum/pectoral girdle length	57%	50-59%	70-79%	70-79%
Hypapophyses of the cervical vertebrae sutured/fused with centum	fused	fused	sutured	sutured
Phalangeal formula of pes	2-3-4-5-4	2-3-4-5-4	2-3-4-5-4	2-3-4-5-4*
Phalangeal formula of manus	2-3-4-5-3	2-3-4-5-3	2-3-4-5-3	2-3-4-5-3*

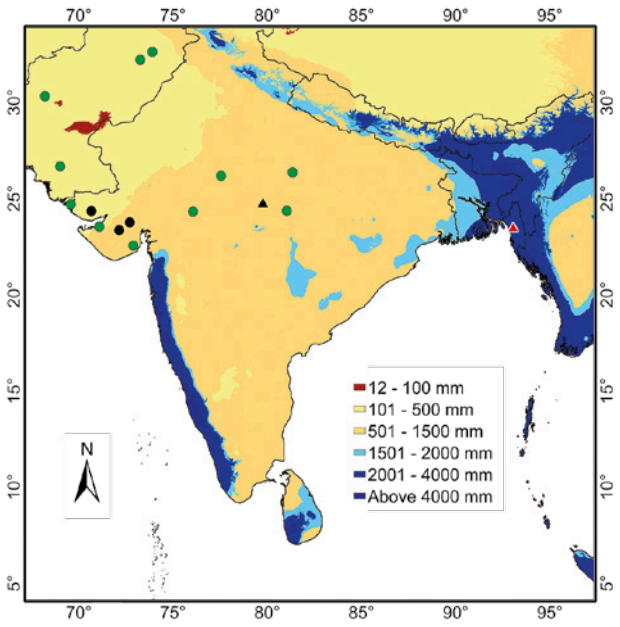

Fig. 7. Rainfall pattern in the India sub-continent and the distribution of *Brachysaura minor*. Red triangle: type locality of *B. minor*, black triangle: location where Blyth's specimens were collected; black circles are place where we collected the samples, green circles previous records.

Notes on distribution

Brachysaura minor was considered to be one of the widespread species of agamid in the Indian subcontinent. However, most recent and confirmed records on the distribution of *B.minor* are from western and central India (see INGLE *et al*. 2012; KHAN & KUMAR 2010). The record of this species from Angul, Odisha by CHAKRABORTY & GUPTA (2009) is that of *Psammophilus* cf. *blanfordianus*. The voucher specimen (ZSI 25833) is deposited at the Zoological Survey of India, Calcutta. We identified this specimen as *Psammophilus* cf. *blanfordianus* based on the small scales on the body and strong fold in the shoulder region. Additionally, *Brachysaura minor* have plate like scales between the eye and above tympanum, which is missing in this specimen. The only record of *B. minor* from eastern part of the subcontinent is the type locality (Chittagong). Previous authors have raised their doubts about the occurrence of *B. minor* in Chittagong (see KHAN & KUMAR 2010). We also suspect this record is erroneous as the habitat at Chittagong is forested with high rainfall compared to open grassland in semi-arid and arid regions of western and central India. While rest of the areas where they occur have mean annual rainfall between 200–1500 mm, Chittagong has more than 2500 mm mean annual rainfall (Fig. 7). Interestingly, the closest sister species to *Brachysaura minor*, *Calotes jerdoni* is distributed in North Eastern India, Myanmar and Bhutan (SMITH 1935, UETZ & HOŠEK 2015).

Discussion

Phylogenetic relationship and morphology

MACEY *et al*. (2000) described six major clades of agamids, which are now considered as subfamilies (PYRON *et al*. 2013). The genus *Calotes*, which belongs to subfamily Draconinae is largely Indian radiation. Our study reveals that the genus *Brachysaura* should be formally placed in the genus *Calotes* (subfamily Draconinae) as *B. minor* is nested within the *Calotes* radiation. Furthermore, *B. minor* also share some prominent morphological characters present in the genus *Calotes* and notable among them is the presence of cardioid structure on the hemipenis, which

is otherwise unique to members of the genus *Calotes* (see Maduwage *et al*. 2008, Maduwage & Silva 2012). Apart from *Calotes*, studies on hemipenial morphology of other agamid lizards suggests that the shape of hemipenis is largely conserved within a genus (Maduwage *et al*. 2008, Maduwage & Silva 2012). Therefore hemipenial morphology is likely to be an important character in diagnosing different agamid genus.

Brachysaura minor have the shortest tail length and fifth toe compared to many other *Calotes*, these morphological difference probably lead to the confusion in the allocation of the genus. The relative length of tail to SVL is remarkably distinct and was used as key for this species (Smith 1935). Most members of genus *Calotes* are arboreal except *Calotes chincollium*, which are reported to be primarily on ground during feeding (Vindum et al. 2003). The reduced fifth toe in *Calotes minor* is most likely an adaptation to exclusive ground dwelling habits. In the Indian sub-continent there are other ground dwelling genera in the subfamily Draconinae, which have reduced or lost its fifth toe. In *Otocryptis,* for example, fifth toe is reduced or rudimentary; while in *Sitana* it is completely lost. Although members of both these genera have relatively long tails. The reduction of tail length is not known in any other members in the subfamily Draconinae. However, in the sister subfamily Agaminae, the following genera *Xenagama, Bufoniceps* and a few species of *Phrynocephalus*, which are exclusively ground dwelling also have reduced tail length. Thus, the characters unique to *Brachysaura minor*, such as short tail and toes, appear to be an adaptation to ground dwelling habit and these characters have evolved convergently in multiple unrelated lineages of agamids.

Overall *B. minor* have greater similarity in osteological characters with the genus *Calotes* than with *Laudakia* or *Agama* (Table 3). *Calotes* have 46–65 caudal vertebrae and the genus *Agama* have 35–45 except *Agama hispida*, which have 20–34 (Moody 1980). Two out of the three species of *Agama* studied by Moody (1980) are saxicolous in habit and one of them "*Agama hispida"* is ground dwelling. *Brachysaura minor* is another ground dwelling agamid, which has reduced number of caudal vertebrae (Table 3).In case of agamids the number of vertebrae does not always correlate with length of the tail instead they are modified long or short and thick (Moody 1980). However, in species like *B. minor* and *A. hispida*, which have relatively short tail, there is evident loss of caudal vertebrae. The genus *Psammophilus* which was earlier considered as *Charasia* have 13 out of 16 osteological characters (included in this study) similar to *Calotes* (Moody 1980) and 11 out of the 16 characters matched with *B. minor*.

Taxonomic implications

Subsuming *Brachysaura* into *Calotes* warrants reassessment of diagnostic characters for the genus *Calotes*. This is because many of the characters used to differentiate between *Pseudocalotes*, *Calotes* and *Bronchocela* are body ratios (Hallermann & Böhme 2000). However *B. minor* is unique among *Calotes* in that it has body ratios that are very different from other *Calotes* spp. This study shows that there are unique hemipenial characters that can be used to diagnose the genus *Calotes*. Though the osteology of agamids is well documented in Moody (1980), it requires extensive revision in the light of new advances in agamid taxonomy. Further studies are required on both hemipenis and osteology to resolve taxonomic ambiguities and for a better understanding of the evolutionary relationships within Draconinae.

The inclusion of genus *Brachysaura* to *Calotes* will not have much taxonomic implications for *Brachysaura minor*, which becomes *Calotes minor* (Hardwicke & Gray 1827). Although Gray (1845) listed *Agama minor* in the synonymy of *Calotes minor* but the latter is a different agamid, presently considered as *Oriocalotes paulus* (Günther 1864, Smith 1935, Ananjeva *et al*. 2011). The specimens of *O. paulus* referred by Gray (1845) under *C. minor* were misidentified and they were not types of the new species. The interpretation by Smith (1935) was wrong, which was corrected by Wermuth (1967). Thus the name *Calotes minor* is not preoccupied and is available for *Brachysaura minor.*

Conclusions

Convergence in morphological characters driven by its ground dwelling habits probably caused confusion in the genus allocation of *Brachysaura minor*. This highlights the need to revise diagnostic characters for the genus *Calotes*. The genus *Trapelus* and *Bufoniceps* are the only Agaminae found in Western India. Therefore our finding also limits the distribution of members of Agaminae to the arid regions (< 500 mm rainfall) of Western India.

Supporting information

Fig. S1. Maximum likelihood tree built using RAG1 sequences.
Fig. S2. Skull of *Brachysaura minor* (CESG466) used to study cranial osteology
Table S1. Sequences used in this study and their GenBank and voucher number.
Table S2. Source for morphometric data on the genus *Calotes* used in figure 4.

Acknowledgements

Ishan Agarwal and Karthik Upadhay for their help in sample collection. Deepak thanks Rufford small grant # 15328-1 for their funds for fieldwork and Department of Biotechnology for DBT-RA fellowship. We also thank MoEF research funding. VG is support-

ed by DAE Outstanding Scientist Grant to Uma. Mohammad Asif for sharing his knowledge on camera-lucida. Shanthanu Kundu and Kaushik Deuti for the photographs of specimens from ZSI, Kolkata. Rohini Balakrishnan for allowing us to use the Camera-lucida facility in her lab. Ulrich Manthey from Germany, Jakob Hallerman from University of Hamburg and Muhammad Rais from University of Karachi and S.R. Ganesh from Chennai Snake Park Trust for providing some of the literature. We also thank Biodiversity hertiage library for the historical literature. Kartik Shanker, S.P. Vijayakumar and Saunak Pal for sharing measurement data for the Western Ghats *Calotes* species. Mukesh Ingle for sharing some data on the Madhya Pradesh *C. minor*. Paul Martyn Cooper and Emily Beech from NHM for finding the original painting of *C. minor* from their collection. Mark Wilkinson from NHM for his support. Ishan Agarwal for his valuable comments. Harry W. Greene for his valuable for his comments on the manuscript. We also thank Rahul Khot and Vithoba Hegde of BNHS for their support. VG thanks Uma Ramakrishnan and Krushnamegh Kunte, NCBS for constant support and encouragement.

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