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REDISCOVERY OF THE HIGH ALTITUDE LAZY TOAD, *Scutiger occidentalis* DUBOIS, 1978, IN INDIA

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Scutiger occidentalis is considered extinct in India. This species has been last reported from India about 40 years ago. We describe a new, genetically confirmed record of *S. occidentalis* obtained in October 2017 from Himachal Pradesh (India), representing the so far southernmost locality for the species. Based on 16 previously known localities of the species, we predicted its potential distribution using a MaxEnt niche model. Of the parameters included in the model, mean diurnal range, land cover and temperature seasonality were the variables with the highest percent contribution for the species. The most suitable habitats for the species were located in Deosai Plateau in Pakistan, mountains around the Kashmir Valley, and along the Dras and Suru river valleys in Ladakh (India).

Keywords: Amphibia; Anura; Megophryidae; distribution; GIS modeling; mtDNA barcoding; Himalaya; Jammu and Kashmir; Himachal Pradesh; extinct species.

INTRODUCTION

The high altitude lazy toads of the genus *Scutiger* Theobald, 1868 belong to the Oriental family Megophryidae Bonaparte, 1850. The genus consists of 22 species which are distributed in southwestern China (including southern and eastern parts of the Tibetan Plateau) and the Himalaya Mountains (Hofmann et al., 2017). Only a single species of the genus (*S. occidentalis*) inhabits northwestern India and adjacent regions of Pakistan. The taxonomic status of the species was long time under discussion. Previously, the species has been reported under various scientific names until Dubois (1978) described it as a separate species but subsequently considered it as a synonym of *Scutiger nyinchiensis* (Dubois, 1987). However, recent genetic data provide evidence that *S. occidentalis* is a valid species (Hofmann et al., 2017).

The first data about occurrence of this species in India were obtained in early 20th century. Annandale (1917) recorded four localities in Kashmir. However, he erroneously assigned the tadpoles found to "*Rana pleskii*." Some years later, Chabanaud (1922) mentioned a specimen of the toad from "Drass" (western Ladakh) under the name "*Bufo andersoni*." Ahmad (1946) found several tadpoles and adults of "*Cophophryne sikkimensis*" in two high-altitude lakes in the Jammu and Kashmir State. Dubois (1978, 1987) described several new localities of *S. occidentalis* in the same state. Finally, Sahi and Duda (1985) and Sahi et al. (1996) reported the species for the vicinities of Kargil town and Khalsi village in Ladakh.

In conclusion, all previously known records of the species in India were reported from Kashmir and Ladakh regions of the Jammu and Kashmir State. The present paper describes the first record of the species from the Himachal Pradesh State of India. Additionally, we attempted to estimate the potential distribution range of the species using GIS modeling.

MATERIAL AND METHODS

DNA extraction and amplification of mtDNA. Total genomic DNA was extracted from dry tissue using the Qiagen DNeasy kit (Qiagen Inc.) following the manufacturer's protocol. We amplified 530 bp of the mitochondrial 16S using the primers and protocols as previ-

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ously described (Hofmann et al., 2017). Amplicons were purified using a QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced in both directions with the same primer pair. Sequence identity was confirmed by using BLASTN suite of NCBI's Web BLAST service (http://blast.ncbi.nlm.nih.gov/Blast.cgi). At least 500 bp of the target 16S sequence and 99% of similarity were used for species identification.

We aligned the new 16S sequence along with available *Scutiger* species and appropriate outgroup sequences retrieved from GenBank (Table 1) using Muscle 3.8.31 as implemented in Mega 6.0 (Thompson et al., 1994; Tamura et al., 2013). The best fitting substitution model (GTR+I+G) for our dataset was determined using jModelTest version 2.1.10 (Darriba et al., 2012) according to the Akaike Information Criterion (AIC). Phylogenetic relationships were calculated based on Bayesian Inference (BI) using MrBayes version 3.2.6 (Ronquist et al., 2012) and run on the CIPRES Science Gateway (Miller et al., 2010) with 20 million generations and four chains, starting with a random tree, sampling trees every 2000th generation until reaching an average standard deviation of split frequencies <0.01. The resulting trees were visualized using FigTree version 1.4.3 (Rambaut, 2016).

Species distribution model. To predict the potential distribution of *S. occidentalis*, we modeled the species' distribution using MaxEnt (ver. 3.3.3k; Phillips et al., 2006). For the contemporary niche predictions, we used 16 localities, comprising own records and previously published data (Table 2; Fig. 1).

TABLE 1. Information on Sequences Used for Molecular Analyses: Taxa, Sample ID or Voucher Numbers, Origin, Sample Locality (Country, Latitude, Longitude, Altitude) and GenBank Accession Number

Taxon	Sample/voucher	Origin	Country	Latitude	Longitude	Alt., m	16S accession No.
S. boulengeri	B04						KR781483
S. boulengeri	JS1507 C1	[1]	China, T	30.3166	91.5166	4400	KY310765
S. boulengeri	A1$AL	[1]	China, T	29.6000	85.7333	5067	KY310760
S. boulengeri	KQ2	[1]	China, Si	30.3666	101.6666	4062	KY310750
S. boulengeri	KQ6	[1]	China, Si	30.3666	101.6666	4062	KY310754
S. boulengeri	KQ7	[1]	China, Si	30.3666	101.6666	4062	KY310755
S. boulengeri	B03	NCBI, ds	Sikkim	28.0263	88.7144	5160	KR781482
S. chintingensis	LC141	[1]	China, Si	30.2333	102.5000	2500	KY310769
S. chintingensis	ROM40460	[2]	China, Si	29.6518	102.9510		EF397270
S. glandulatus	SC1$2014	[1]	China, Si	31.6666	99.7166	3457	KY310770
S. glandulatus	SH150557	[1]	China, Si	29.7833	100.4166	3713	KY310774
S. glandulatus	CIBXM1188	[2]	China, Si	29.1890	100.1101		EF397274
S. liupanensis		NCBI, ds					KX352261
S. mammatus	CIBXM972	[2]	China, Si	30.0325	101.4749		EF397279
S. muliensis	IOZCAS3638	[2]	China, Si	27.3418	101.5403		EF397277
S. nepalensis	A2014	[1]	Nepal	29.3500	82.1500	3285	KY310776
S. nepalensis	Scut8	[1]	Nepal	29.3500	82.3833	4450	KY310790
S. nepalensis	A8-2012	[1]	Nepal	28.7000	82.9166	3340	KY310778
S. nepalensis	A9-2012	[1]	Nepal	28.6000	83.0166	3250	KY310779
S. nepalensis	A14-2012	[1]	Nepal	28.5000	83.0333	3073	KY310784
S. nepalensis	A15-2012	[1]	Nepal	28.5000	83.1166	2993	KY310785
S. ningshanensis		NCBI					KX352260
S. occidentalis	PK1	[1]	Pakistan	34.9833	75.2500	4100	KY310793
S. occidentalis	SL1Tra	This study	India	32.9517	76.1904	2896	MG923583
S. sikkimensis	Ne5-2013	[1]	Nepal	27.5666	86.8000	2900	KY310802
S. sikkimensis	Ne11-2013	[1]	Nepal	27.6500	86.6000	3900	KY310806
S. sikkimensis	JS140523	[1]	Nepal	27.6333	87.2166	3000	KY310797
S. sikkimensis	B06	NCBI, ds	India	27.8117	88.5520	3273	KR781485
S. tuberculatus	CIBXM988	[2]	China, Si	28.5280	102.5114		EF397278
O. omeimontis							EU180886
O. chuanbeiensis							EU180887

Note. Alt: altitude; ds: direct submission; Lat: latitude; Long: longitude; O.: *Oreolalax*; S.: *Scutiger*; Si: province Sichuan; T: Tibet. [1] Hofmann et al. (2017); [2] Fu et al. (2007).

Altitude and 19 bioclimatic layers were extracted from the WorldClim 1.4 database (http://www.worldclim.org). Further layers were obtained from the following databases: Global Aridity and Potential Evapo-Transpiration (http://www.cgiar-csi.org) and GlobCover 2009 (Global Land Cover Map; due.esrin.esa.int/globcover). In addition, 14 layers with various metrics quantifying spatial heterogeneity of global habitat based on the textural features of Enhanced Vegetation Index (EVI) imagery and the global Percent tree coverage were downloaded from EarthEnv (http://www.earthenv.org/texture.html) and Github (https://github.com/globalmaps/gm_ve_v1), respectively. To consider topography in the model, four landscape layers (aspect, exposition, slope, and terrain roughness index) were calulated with QGIS 2.18.1 (http://www.qgis.org).

To eliminate predictor collinearity prior to generating the model, we calculated Pearsons's correlation coefficients for all pairs of bioclimatic variables using the ENMTools (Warren et al., 2010). We excluded the variable of a correlated pair with $|r| > 0.8$ that we considered to be the less biologically important of the two, based on known preferences of *S. occidentalis*. The resulting dataset contained six bioclimatic variables: Bio1 (annual mean temperature; °C×10), Bio2 (mean diurnal range; °C×10), Bio4 (temperature seasonality; standard deviation ×100), Bio15 (precipitation seasonality; CV), Bio16 (precipitation of wettest quarter; mm), and Bio19 (precipitation of coldest quarter; mm). We used a jackknife analysis for estimating the relative contributions of the variables to the MaxEnt model and applied a mask that extends from 30 to 37° N and 71 to 81° E.

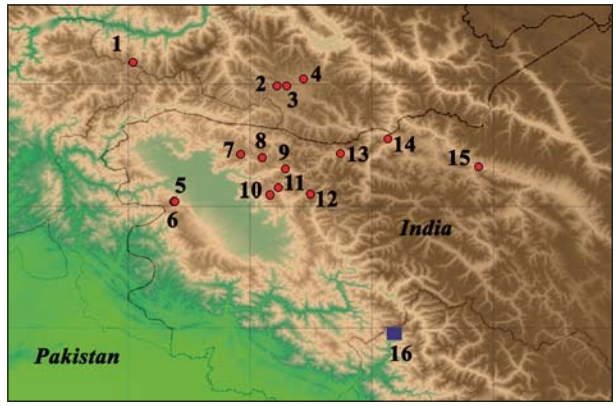

Fig. 1. The map of distribution of *Scutiger occidentalis*. Numbers of localities are given in Table 2. The new record from Trakar Rani Kot in Himachal Pradesh (India) is indicated by a blue square.

A total of 27 variables (30 arc-sec resolution) were implemented in the final model. We used 70% of the occurrence localities as training data, and the remaining 30% were reserved for testing the resulting models. Model performance was measured using the Area Under the Curve (AUC) derived from the Receiver Operating Characteristic (ROC) plots. The plots represent a model's ability to discriminate species locations from pseudo-absences by plotting sensitivity against 1 — specificity. AUC values range from 0.5 to 1.0, with 0.5 indicating no greater fit than expected by chance and 1.0 indicating a perfect model fit. Models with test AUC values above 0.90 are considered as very good (Swets, 1988). To properly parameterize the model, we evaluated the perfor-

TABLE 2. List of localities of *Scutiger occidentalis*.

No.	Locality	Country	Coordinates	Altitude, m	Reference
1	Babusar, 4.2 km N	Pakistan	35.1800° N 74.0400° E	~3300	Hofmann et al., 2017
2	Sheosar Lake	Pakistan	34.9833° N 75.2200° E	~4100	Hofmann et al., 2017
3	Deosai Plateau	Pakistan	34.9833° N 75.3000° E	4300	Ficetola et al., 2010
4	Deosai Plateau	Pakistan	35.0400° N 75.4400° E	4300	Hofmann et al., 2017
5	Khilanmarg	India	~34.0400° N 74.3700° E	2680 – 2850	Dubois, 1978
6	Gulmarg	India	~34.0400° N 74.3833° E	2690	Dubois, 1978
7	Ganderbal Lake	India	~34.4250° N 74.9250° E	3567	Annandale, 1917
8	Kreshen Sar Lake	India	~34.3971° N 75.1004° E	3677	Annandale, 1917
9	Shukdhari	India	~34.3019° N 75.2894° E	2920 – 3200	Dubois, 1978
10	Chandra Sar Lake	India	~34.0931° N 75.1612° E	3901	Ahmad, 1946
11	Lidarwart	India	~34.1500° N 75.2300° E	2743	Annandale, 1917
12	Shesh Nag Lake	India	~34.0943° N 75.4971° E	3658	Ahmad, 1946
13	Dras	India	~34.4300° N 75.7440° E	~3082	Chabanaud, 1922
14	Kargil	India	~34.5500° N 76.1333° E	2680	Sahi, Duda, 1985
15	Khalsi	India	~34.3188° N 76.8776° E	2750	Sahi, Duda, 1985
16	Trakar Rani Kot	India	32.9517° N 76.1904° E	2896	Present paper
17	Nagabera	India	—	3050 – 3200	Annandale, 1917

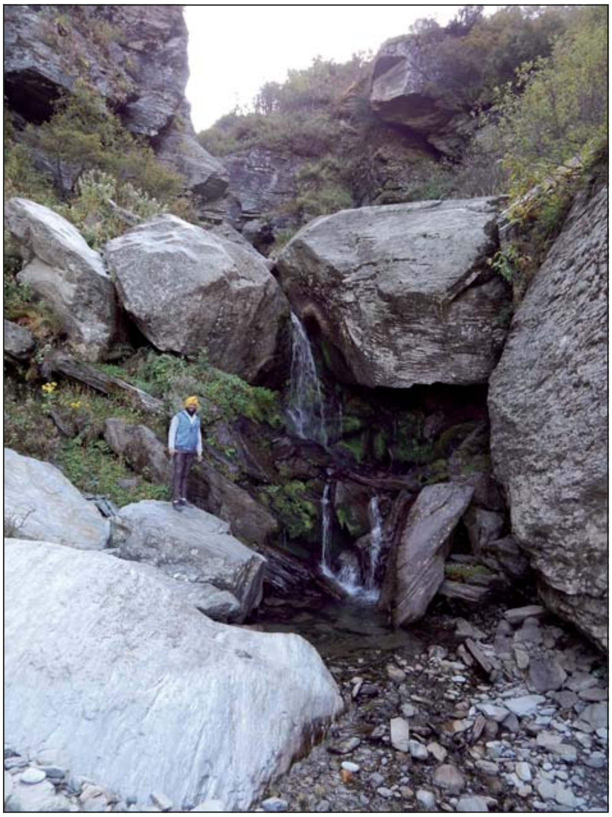

Fig. 2. The habitat of *Scutiger occidentalis* in Trakar Rani Kot (Himachal Pradesh, India).

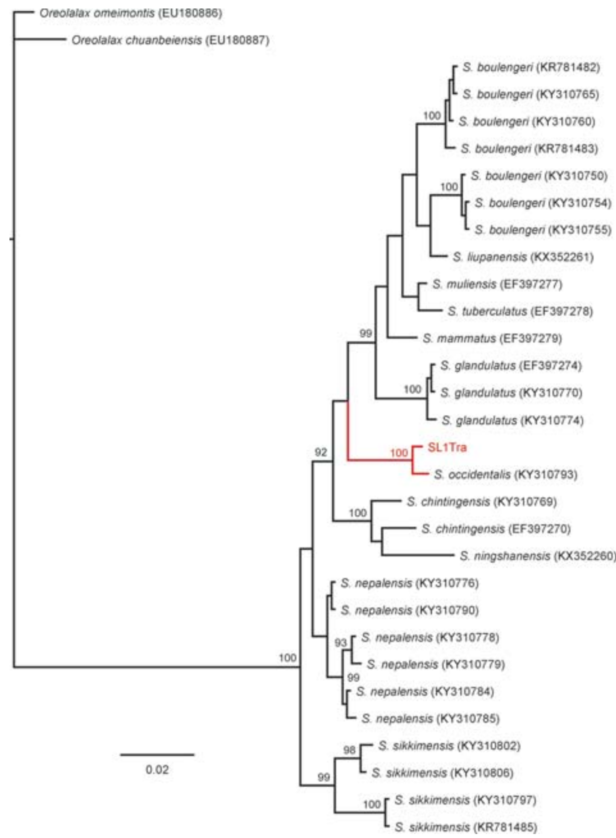

Fig. 4. The Bayesian Inference consensus tree for species of the genus *Scutiger*. The sample of *S. occidentalis* from Trakar Rani Kot (Himachal Pradesh, India) is indicated in red.

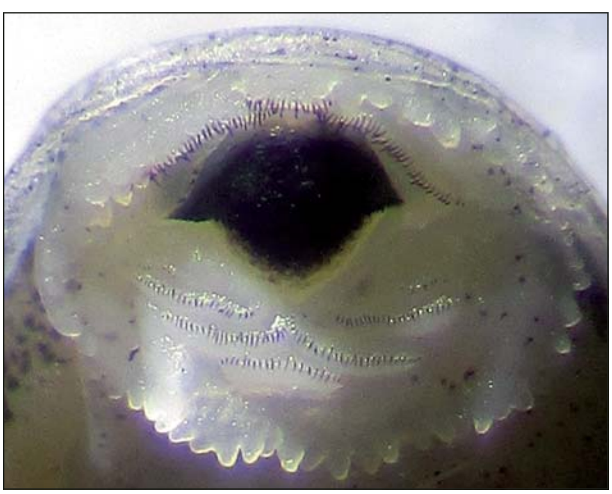

Fig. 3. The oral disc and mouth of a tadpole of *Scutiger occidentalis* from Trakar Rani Kot (Himachal Pradesh, India).

mance of various combinations of ten regularization multipliers (from 0.5 to 5, in increments of 0.5). The best-fit models were parameterized with a regularization multiplier of 0.5. We used the default auto feature setting within the MaxEnt software to generate the model.

RESULTS AND DISCUSSION

In October 7, 2017, three of the authors (S. N. Litvinchuk, D. A. Melnikov and L. J. Borkin) moved by car from Bairagarh village to the Sach Pass (4390 m above sea level) in Chamba District, the north-western part of Himachal Pradesh State, India (Western Himalaya, the southern slope of Pir Panjal Range). At 8:40 of a local time, in a sparse mixed forest near Trakar Rani Kot farm (32.951734° N 76.190370° E) we stopped to make photos. In a pool under a waterfall with diameter of about 3 m and a maximal depth of 0.5 m (Fig. 2), a single small tadpole was found. The temperature of the water in the pool was 7.3°C. No other tadpoles or adult amphibian, fish, and reptile species were observed at this place.

The specimen had a snout-vent length of 8.9 mm, a total length of about 22.5 mm, and it was at developmental stage 25 (Gosner, 1960) with undeveloped oral disc

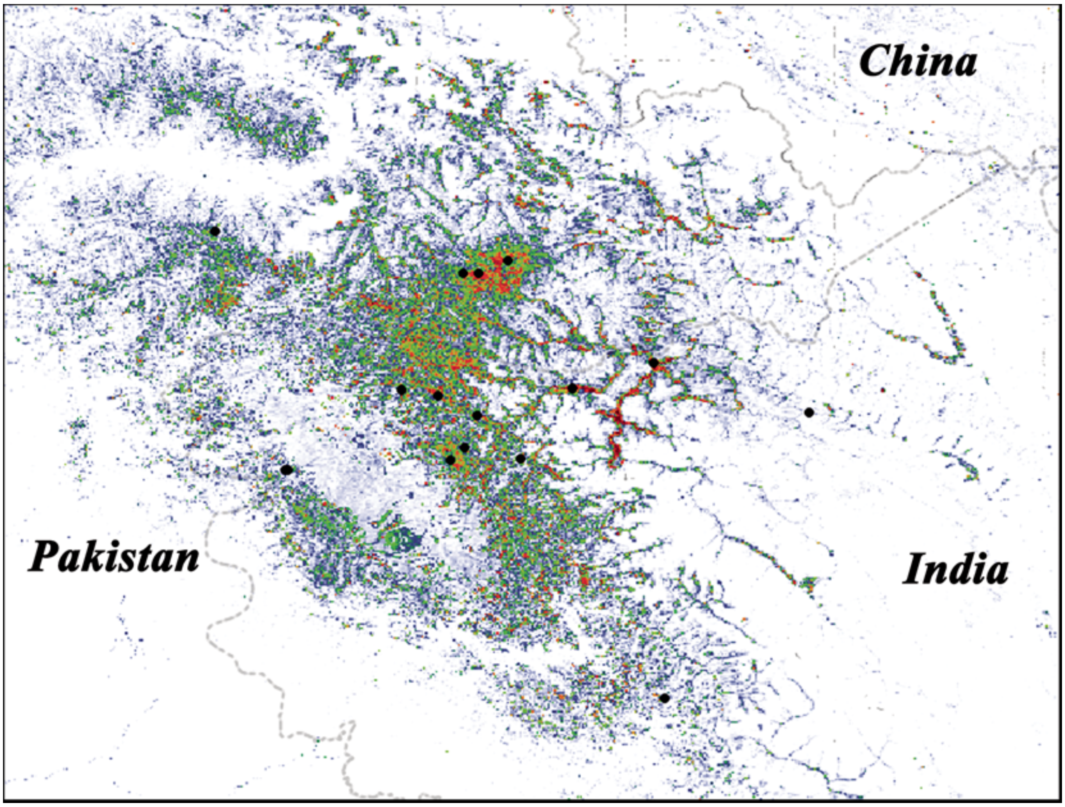

Fig. 5. The predicted potential distribution model of *Scutiger occidentalis* generated with MaxEnt. Most suitable regions are indicated by orange and red colors (probability of occurring of the species are 0.5 – 1.0); moderately suitable are in green and blue (0.1 – 0.5); and little or unsuitable are in white (0 – 0.1). Localities of the species are given as black circles.

and mouth (Fig. 3); the upper row of teeth on the lower lip was incompletely formed. In general, the morphology of the oral disc and mouth of our tadpole was similar to larvae of *S. occidentalis* described by Annandale (1917) and Hofmann et al. (2017).

Our partial 16S gene sequence (accession number MG923583) was 530 bp in length. When blasted against sequences in GenBank there was 100% query cover and 99% pairwise identity of the *S. occidentalis* sequences to accession numbers KY310793- KY310796 (note that *S. occidentalis* is listed in GenBank as *S. nyingchiensis*, but see Hofmann et al., 2017). The BI consensus tree (Fig. 4) was poorly resolved due to the limited molecular data. However, our new sample from Himachal Pradesh (SL1Tra) clusters consistently together with *S. occidentalis* with highest posterior support.

All defined records of *S. occidentalis* are summarized in Table 2 and Fig. 1. According to these data, *S. occidentalis* inhabits high-mountain regions in northern Pakistan and India. In Pakistan, the lazy toad had been reported from four localities in Gilgit-Baltistan Province (3300 – 4300 m a.s.l.). In India, the species is historically known from 12 localities between 2680 and 3901 m a.s.l. in Jammu and Kashmir, while in Himachal Pradesh the species hasn't been found until our observation near Trakar Rani Kot farm at 2896 m a.s.l. This new record represents the so far southernmost locality for *S. occidentalis*.

The MaxEnt model for the species had robust evaluation metrics ($AUC_{test} = 0.938 \pm 0.015$) and showed significance for the binomial omission test, indicating a good performance of the model. The predicted potential niche model under the current climate conditions is shown in Fig. 5. Of the parameters included in the model, mean diurnal range, land cover and temperature seasonality were the variables with the highest percent contribution for the species (29, 18, and 17%, respectively). The most suitable habitats for the species were in Deosai Plateau in Pakistan, mountains around the Kashmir Valley, and along the Dras and Suru river valleys in western Ladakh (India). Although the new record in Himachal Pradesh (No. 16 in Fig. 1) is located in an environment which seems to be less suitable for the species (occurrence probability = 0.15), the probability of the species' occurrence increased substantially (0.46) in the region about 1 km to the west.

The IUCN red list map for *S. occidentalis* (corresponds to the western part of range of *S. nyingchiensis*; Fei et al., 2004) shows a broad distribution range of the species extending to central Nepal. According to our MaxEnt model, the limits of distribution of *S. occidentalis* to the east of its range do not exceed the northwestern part of Himachal Pradesh.

In conclusion, while *S. occidentalis* was recorded from Jammu and Kashmir (India) 40 years ago (Dubois, 1978; Sahi and Duda, 1985; Biju, 2017) and it was never mentioned for Himachal Pradesh (Singh, 1982; Mehta, 2005; Sharma, Deuti, 2014; Sharma, Sidhu, 2016; Litvinchuk et al., 2017), we rediscovered the species from India in 2017 and provide the first record of it from western Himachal Pradesh.

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