

**The Data Deficient *Leptobrachella nokrekensis* is a  
junior synonym of the supposedly range-restricted  
and Critically Endangered *Leptobrachella khasiorum*  
(Amphibia: Anura: Megophryidae)**

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1   **The Data Deficient *Leptobrachella nokrekensis* is a junior synonym of the supposedly**  
2   **range-restricted and Critically Endangered *Leptobrachella khasiorum* (Amphibia:**  
3   **Anura: Megophryidae)**

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16   **Abstract.**

17   Based on a molecular phylogeny using 16S ribosomal RNA gene fragment and an examination  
18   of the external morphology of topotypic specimens we demonstrate that *Leptobrachella*  
19   *nokrekensis* syn. nov. from Garo hills is a junior synonym of *Leptobrachella khasiorum*. These  
20   data indicate that *Leptobrachella khasiorum* which was previously considered restricted to the  
21   Khasi Hills and Critically Endangered is more widespread than previously known. Genetic  
22   evidences also suggests that *L. tamdil* from Lushai hills may also be conspecific with *L.*  
23   *khasiorum*, warranting further investigations. A reassessment of its threat status is required  
24   based on this taxonomic revision and the consequent extension of *L. khasiorum*'s distribution  
25   and elevational range.

26   **Keywords.** 16S rRNA, Data Deficient, Endemism, Amphibians, IUCN Red List, Eastern  
27   Himalayas, Taxonomic revision, Threat status reassessment, Darwinian shortfall.

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## INTRODUCTION

31        The Khasi, Garo and Lushai hills of Northeast India are disconnected mountain ranges  
32        with interesting biogeographical connections to the Eastern Himalayas, the Brahmaputra Valley  
33        and the Indo-Malayan region including the Malayan Peninsula (Olson and Dinerstein, 2002;  
34        Pawar et al., 2007; Badavath and Sahoo, 2025). The region's amphibian fauna has been studied  
35        since the mid-19th century, yet many taxonomic revisions are ongoing as recent collections  
36        continue to provide new insights into species boundaries and diversity (Kamei et al., 2012; Biju  
37        et al., 2019; Saikia & Sinha 2019; Mahony et al., 2020; Patel et al., 2021; Saikia et al., 2022;  
38        Boruah et al., 2023; Naveen et al., 2023). There are a few examples of congeneric herpetofaunal  
39        diversity that are distinct between these ranges, probably due to the geographic disconnectivity  
40        (e.g., Lalronunga et al., 2021; Naveen et al., 2023; Mirza et al., 2024) while many others are  
41        known to occur across them (e.g., Siammawii et al., 2021; Muansanga et al., 2022; Naveen et  
42        al., 2025).

43        Four species of *Leptobrachella* Cope, 1865 are known from India, of which the three  
44        species dealt with in this study were described within a short span between January and June  
45        2010, from the Khasi, Garo, and Lushai Hills. *Leptobrachella khasiorum* (Das, Tron, Rangad,  
46        and Hooroo, 2010), originally described as *Leptolalax khasiorum* from Mawphlang Sacred  
47        Grove in the Khasi Hills is believed to be endemic to just this region. Consequently, it is  
48        categorized as Critically Endangered due to its extremely narrow Extent Of Occurrence.  
49        *Leptolalax tamdil* (Sengupta, Sailo, Lalremsanga, Das, and Das, 2010) was described from the  
50        nearby Tamdil wetlands in Mizoram. This species was subsequently transferred to the genus  
51        *Leptobrachella* and reported from a few other locations in central Mizoram, as well as from

52 another more northeastern location in Manipur (Decemson et al., 2021). A third species,  
53 described from the Garo hills, *Leptobrachium nokrekensis*, later transferred to the genus  
54 *Lepobrachella*, although claimed to be described in “2009” by Mathew and Sen (2009), is now  
55 considered *Leptobrachella nokrekensis* (Mathew and Sen, 2010 “2009”), since Das and Deuti  
56 (2011) convincingly argued that the name was only made available on June 3, 2010, the official  
57 date of distribution of this printed publication.  
58 *Leptobrachella* is one of the most diverse genera in the family Megophryidae Bonaparte, 1850  
59 with 117 species distributed across southern China, northeastern India, Myanmar, Thailand,  
60 Vietnam, Malaya, Borneo, and the Natuna Islands (Frost 2025). The members of this genus  
61 exhibit a high degree of localised endemism and morphological overlap (Nguyen et al., 2021;  
62 Wu et al., 2025). But despite their cryptic morphology, new species continue to be identified,  
63 based on evidence from molecular analyses (Lin et al., 2022; Hoang et al., 2024; Luo et al.,  
64 2025; Wu et al., 2025). However, in the case of the northeastern Indian species, *Leptobrachella*  
65 *khasiorum*, *Leptobrachella nokrekensis*, and *Leptobrachella tamdil*, even though all of them  
66 exhibit a high degree of morphological similarity, none of their original descriptions included  
67 molecular data (Das et al., 2010; Mathew and Sen, 2010 “2009”). In the current study, the  
68 taxonomic validity of *Leptobrachella nokrekensis* is re-examined, based on fresh topotypical  
69 material collected from the Garo and Khasi hills, and further studies on *Leptobrachella tamdil*  
70 from Mizoram to assess its status is recommended.

## 71 MATERIAL AND METHODS

72 *Sampling*  
73 Specimens were collected from two locations of the northeast Indian state of Meghalaya:  
74 Sakalgre Village, Garo hills (25.515992, 90.381045; 925 m asl): Three specimens—one adult

75 male (PU RSN 23) and two adult females (PU RSN 24, PU RSN 34), collected by RSN from  
76 a stream near the village in October 2024.

77 Near Mawphlang Sacred Grove, Khasi hills (25.436459, 91.759246; 1575 m asl): Two  
78 specimens—one adult male (PU RSN 32) and one adult female (PU RSN 33), collected by  
79 RSN from a stream flowing out of the grove in October 2024.

80 Specimens were collected under the following permits (No. WC/Research/157/608). They  
81 were photographed in life before being euthanized using approximately 0.10 ml of a 20%  
82 benzocaine solution applied to the ventral surface. Liver tissues were extracted from freshly  
83 euthanized specimens for molecular analyses and stored in 99% molecular-grade ethanol. The  
84 specimens were then fixed in 10% buffered formalin and subsequently stored in 75% ethanol.

85 All specimens were deposited in the collections of the Department of Ecology and  
86 Environmental Sciences, Pondicherry University (PU).

#### 87 *Measurements*

88 Morphological data from fixed specimens were measured to the nearest 0.02 mm with  
89 INSIZE digital callipers. The following measurements were taken: Snout-Vent Length (SVL);  
90 Axilla-to-Groin Length (AGL); Mid-Body Width (MBW); Head Width (HW) (measured at  
91 the angle of the jaws); Head Length (HLD) (from posterior end of the mouth snout tip);  
92 Nostril-to-Snout Distance (NS); Internarial Distance (IN); Maximum Upper Eyelid Width  
93 (UEW); Eye Diameter (ED); Interorbital Distance (IO) (shortest distance between the upper  
94 eyelids); Eye-to-Nostril Distance (EN); Tympanum Diameter (TYD); Tympanum-to-Posterior  
95 Corner of Eye Distance (TE); Upper Arm Length (UAL); Forearm Length (FAL) (previously  
96 Forelimb Length) (measured from the elbow to the base of the outer palmar tubercle); Palm  
97 Length (PAL) (measured from the base of the outer palmar tubercle to the tip of the third  
98 finger); Thigh Length (THL); Shank Length (SL) (approximated by measuring the crus); and,

99 Foot Length (FL) (measured from the base of the inner metatarsal tubercle to the tip of the  
100 fourth toe).

101 *Molecular analysis*

102 Total genomic DNA was extracted from two specimens of *Leptobrachella* from Meghalaya  
103 (PU RSN 33 and PU RSN 24) with a DNA extraction and purification kit, following the  
104 manufacturer's protocols. A fragment of the 16S rRNA gene was amplified using the primers  
105 16sAR-L (5' CGCCTGTTATCAAAACAT-3') and 16sBR-H  
106 (5'CCGGTCTGAACTCAGATCACGT 3') respectively (Kocher et al., 1989). Amplifications  
107 were performed in an Applied Bio Systems Veriti 96 well thermal cycler: 20  $\mu$ l reactions with  
108 4  $\mu$ l of 5X Phusion HF buffer, 0.4  $\mu$ l of 10mM dNTP, 0.2  $\mu$ l of Phusion DNA Polymerase,  
109 0.1  $\mu$ l each of forward and reverse primers, 2.0  $\mu$ l of DNA template and 13.2  $\mu$ l of nuclease  
110 free water with the following procedure: initial denaturation of DNA at 95°C for 5 min, 35  
111 cycles of: denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for  
112 1 min and at last, final extension at 72°C for 10 min. The amplicon was checked by running it  
113 through an agarose gel electrophoresis for a clear band of the desired region in the amplified  
114 PCR product. The amplified PCR product was purified and sequenced commercially at  
115 Barcode BioSciences Pvt.Ltd (BBS), Bangalore, India. The new sequences were then  
116 checked on the Basic Local Alignment Search Tool, BLAST (The National Centre for  
117 Biotechnology Information) (Altschul et al. 1990) to verify their approximate identity.

118 *Phylogenetic analysis*

119 The new topotypic sequences of *Leptobrachella khasiorum* and *Leptobrachella nokrekensis*  
120 were assembled along with 52 other congeners from the Indo-Burma, Indo-China and  
121 southwestern China regions, with *Leptobrachium sylheticum* and *Xenophrys major* as the

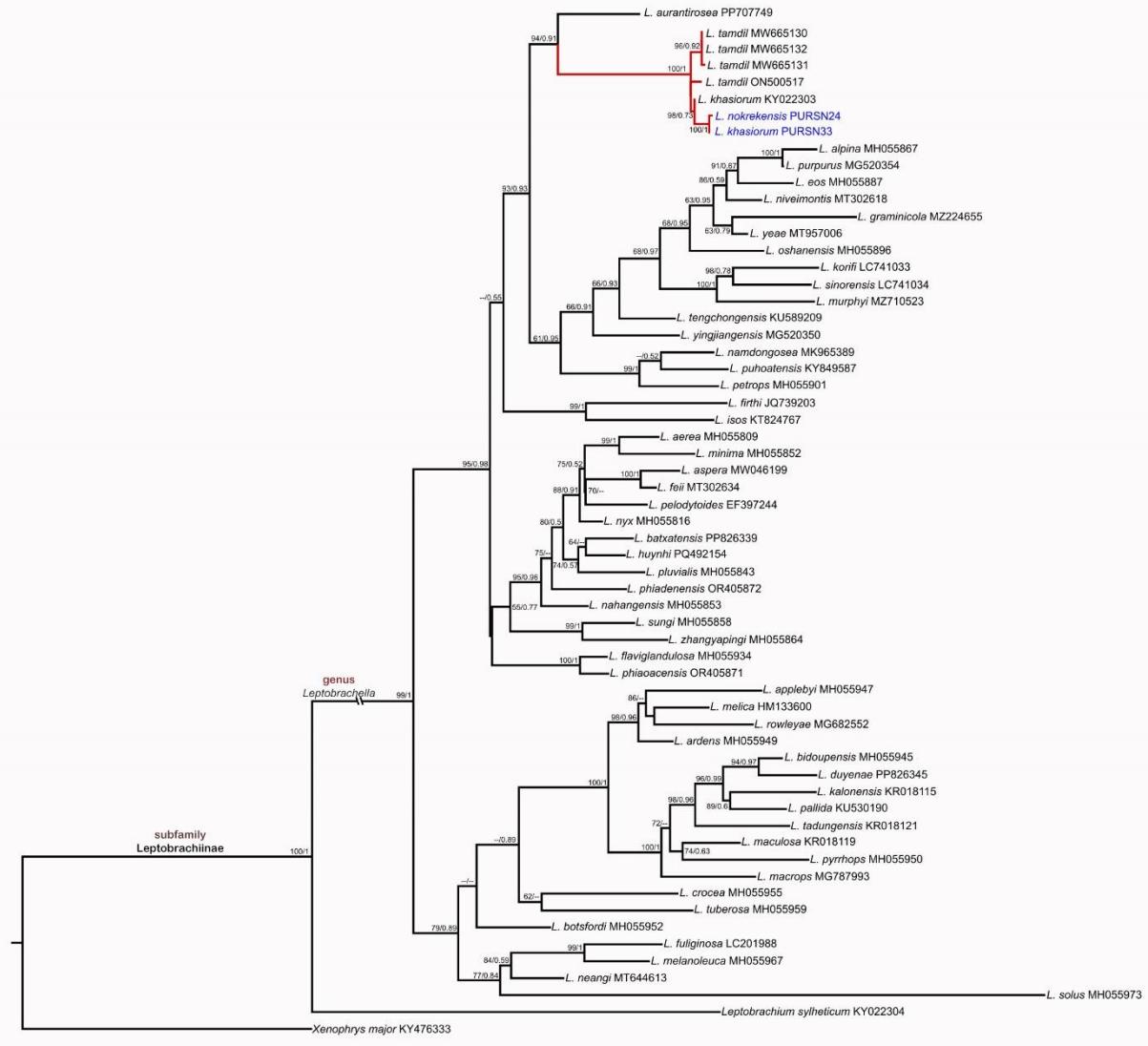
122 outgroups (see Fig. 1). The single existing sequence from the type locality of *Leptobrachella*  
123 *khasiorum* and all available homologous sequences of *Leptobrachella tamdil* were also added  
124 to the dataset. The resultant dataset of 61 sequences were aligned with Muscle and optimized  
125 manually, in MEGA X (Kumar et al., 2018). This alignment was then used to determine the  
126 uncorrected pairwise genetic distances between the samples with MEGA X. For the  
127 phylogenetic analysis, the best-fit model of nucleotide substitution was selected in  
128 jModelTest2 (Darriba et al., 2012). MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) was  
129 used to perform the Bayesian analysis under the GTR+I+G model. Two independent  
130 analyses, each consisting of four Metropolis-coupled Markov chain Monte Carlo (MCMC)  
131 chains, were run for 20 million generations, with parameters sampled every 1,000  
132 generations. Convergence was evaluated by ensuring that the average standard deviation of  
133 split frequencies fell below 0.01 and that potential scale reduction factors approached 1.0.  
134 Stationarity and effective sample sizes (ESS) were monitored within MrBayes. The first 25%  
135 of sampled trees were discarded as burn-in following Huelsenbeck et al. (2001), and clade  
136 support was assessed using Bayesian posterior probabilities (BPP), with values  $\geq 0.95$   
137 considered strong (Leaché and Reeder, 2002). Maximum-likelihood (ML) analyses were  
138 carried out in IQ-TREE v1.6.12 (Nguyen et al., 2015) under the GTR+F+I+G4 model.  
139 Branch support was estimated using 10,000 ultrafast bootstrap (UFB) replicates (Hoang et al.,  
140 2018). Nodes with UFB  $\geq 95$  were considered strongly supported (Minh et al., 2013).

141

## RESULTS

142 The Bayesian Inference and Maximum Likelihood trees obtained were similar in topology  
143 and largely congruent with existing recent large scale phylogenies of *Leptobrachella*. The  
144 relationships between the focal group, comprising *Leptobrachella khasiorum*, *L. nokrekensis*  
145 and *L. tamdil*, were well resolved and the new sequences of *L. khasiorum* and *L. nokrekensis*

146 grouped together with the sequence of *L. khasiorum* by Mahony et al., 2017 from the type  
 147 locality. The Indian taxa formed a strongly supported monophyletic clade (UFB 100; BPP 1)  
 148 which showed a sister relationship to a recently described species *L. aurantirosea*. The  
 149 uncorrected pairwise genetic distance at the 16S mitochondrial gene between the new  
 150 samples from this study and the available sequence of *L. khasiorum* from the GenBank was  
 151 0.2 – 1.3%. These shallow divergences fall within the range typically considered conspecific  
 152 among members of this genus and anurans as a whole for the 16S mtDNA gene.



154 **Fig 1.** Maximum Likelihood tree based on the 16S mtDNA gene for *Leptobrachella* species  
155 of India studied here and congeners from nearby regions. The clade containing the Indian  
156 taxa is highlighted in red, and newly collected topotypic samples are shown in blue. Branch  
157 support values represent Ultrafast Bootstrap Support (UFB > 50%) and Bayesian Posterior  
158 Probabilities (BPP > 0.50), respectively.

159 Furthermore, morphological characteristics were similar among newly collected specimens.  
160 Based on comparative examination of specimens of *L. khasiorum* with the original  
161 descriptions of *L. nokrekensis* provided in Mathew and Sen, (“2009” 2010), Das and Deuti,  
162 (2011) point out, the following differences between these two species, *L. nokrekensis* (vs. *L.  
163 khasiorum*) “*reduced tympanum, being less than half orbit diameter (vs. half orbit diameter);  
164 area around pectoral region pale (vs. with dark pattern); paired red tubercles anterior to  
165 tympanum (vs. absent); loreal region concave (vs. sloping); and dorsum with longitudinal  
166 folds (vs. absent).*”.

167 In contrast, the series of topotypic *Leptobrachella* specimens collected and examined in this  
168 study, including adult specimens from both the Garo and Khasi Hills, consistently show a  
169 tympanum size greater than half the orbit diameter, an absence of longitudinal folds on the  
170 dorsum, and a consistently concave loreal region in all specimens. Other differences in  
171 coloration and tuberculation noted by Das and Deuti, (2011), these traits are now known to be  
172 highly polymorphic in this genus and are not always reliable for species delimitation (see  
173 intraspecific variation section in Nguyen et al., 2021; Lin et al., 2022; Hoang et al., 2024; Luo  
174 et al., 2025; Wu et al., 2025).

175 Based on these morphological and molecular evidences from the topotypes, it’s evident that  
176 *Leptobrachella khasiorum* and *L. nokrekensis* are not two distinct species. The remaining  
177 question then is nomenclatural priority. Although *L. nokrekensis* was initially believed to

178 have been described earlier, Das and Deuti, (2011) clarified that its official date of  
179 availability was on June 3, 2010. Thus, the nomen *L. khasiorum*, made available on January  
180 10, 2010, in Das et al., (2010), is the first available among the two. Therefore, *L. khasiorum*  
181 is the valid nomen for this species, and *Leptobrachella nokrekensis* syn. nov. is its junior  
182 synonym.



183



184

185 **Fig 2a.** *Leptobrachella khasiorum* (PU RSN 33) from Mawphlang, Khasi hills



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187

188 **Fig 2b.** *Leptobrachella nokrekensis* syn. nov. (PU RSN 24) from Sakalgre, Garo hills.

189 The preliminary molecular data presented here suggest that *L. tamdil* too is a probable junior  
190 synonym of *Leptobrachella khasiorum* (uncorrected p-distance 1.0 – 2.6%). However, this  
191 warrants further studies, as the current study was unable to examine any fresh specimens  
192 from Mizoram or the specimens associated with the sequences used from GenBank in this  
193 study and also the available GenBank sequences are not from the type locality but from other  
194 localities in Mizoram and Manipur. Therefore, this is left unresolved here for further studies  
195 to resolve.

196

## DISCUSSION

197 With the results presented here, *Leptobrachella khasiorum*, previously thought to be  
198 Critically Endangered due to its narrow endemic distribution range, restricted to a small  
199 forested area in and around the Mawphlang Community Reserve, is now known to occupy a  
200 substantially wider range, including the known occurrence locations of *L. nokrekensis* syn.

201 nov., across Nokrek National Park. In addition to its previously known occurrences in the  
202 Garo Hills, this species was recorded near Sakal Aduma (25.524°N, 90.342°E; 980 m asl) and  
203 near Tura Peak (25.515°N, 90.232°E; 820 m asl) during this study. In addition to this,  
204 Chandramouli et al. (2022) reported a specimen of *Leptobrachella* (referred to it as  
205 *Leptobrachella* cf. *khasiorum*, (SACON VA 115—an unsexed subadult collected by P.  
206 Karthik), from Jirang (25.945°N, 91.567°E, 1280 m asl), approximately 50 km from  
207 Mawphlang in a straight line. It is likely that this specimen also belongs to the same species,  
208 as the location falls within the now known elevational range of *L. khasiorum* and is relatively  
209 close to Mawphlang. With these new records, the updated Extent of Occurrence for this  
210 species is 4,027 km<sup>2</sup> across an elevational range of 800 m asl to 1,600 m asl. In the locations  
211 where this species was recorded during this study, it was found to be abundant. The species is  
212 also likely distributed across several other protected areas, in addition to Nokrek National  
213 Park and Mawphlang Sacred Forest. Based on this distributional information, the species  
214 qualifies for being downlisted from Critically Endangered under the IUCN Red List criteria  
215 (see IUCN 2012). This could still be an underestimation of the distribution range of this  
216 species and further survey efforts across these regions, as well as the clarification of the  
217 taxonomic status of *Leptobrachella tamdil* is required to accurately define the distribution  
218 and conservation status of *Leptobrachella khasiorum*.

219 In addition to these three species, Northeast India also harbours another Critically  
220 Endangered *Leptobrachella*, *Leptobrachella lateralis* (Anderson, 1871), which was originally  
221 thought to be from Bhamo but has now been neotyped from the Naga Hills. This species  
222 remains poorly documented, with no data on its phylogenetic position or natural history, and  
223 continues to be known with confidence only from the neotype locality, with no subsequent  
224 confirmed records elsewhere. We did not survey its range during the present study. Based on  
225 general biogeographic patterns, we infer that this species is unlikely to be closely related to

226 the *Leptobrachella* populations of the Khasi, Garo, and Lushai Hills, as the Naga Hills are  
227 comparatively less connected to these ranges. Further surveys are therefore necessary to  
228 clarify its distribution and taxonomic status.

229

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237

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335 **Table 1.** Genetic divergences (uncorrected p-distance) between specimens identified as  
 336 *Leptobrachella khasiorum*, *Leptobrachella nokrekensis*, and *Leptobrachella tamdil*.

	Location	1	2	3	4	5	6	7
<i>L. nokrekensis</i>	Garo							
This Study	Hills							
<i>L. khasiorum</i>	Khasi		0.0026					
This Study	Hills							
<i>L. khasiorum</i>	Khasi		0.0131	0.0105				
KY022303	Hills							
<i>L. tamdil</i>								
ON500517	Mizoram	0.0236	0.0209	0.0105				
<i>L. tamdil</i>								
MW665130	Mizoram	0.0236	0.0209	0.0105	0.0157			
<i>L. tamdil</i>								
MW665131	Mizoram	0.0262	0.0236	0.0131	0.0183	0.0026		
<i>L. tamdil</i>								
MW665132	Mizoram	0.0236	0.0209	0.0105	0.0157	0.0000	0.0026	-

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341 **Table 2.** Morphometric measurements (in mm) of *Leptobrachella khasiorum* and  
 342 *Leptobrachella nokrekensis* specimens examined in this study.

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Voucher number	PU RSN 23	PU RSN 24	PU RSN 34	PU RSN 32	PU RSN 33
Location	Sakalgre Village, Garo Hills	Sakalgre Village, Garo Hills	Sakalgre Village, Garo Hills	Near Mawphlang Sacred Grove, Khasi Hills	Near Mawphlang Sacred Grove, Khasi Hills
Sex	Male	Female	Female	Male	Female
SVL	33.1	32.25	30.65	27.85	28.05
AGL	15.7	14.14	12.18	10.6	10.04
MBW	12.34	11.78	10.02	8.06	8.37
HW	11.06	11.3	10.85	9.31	9.54
HLD	11.31	11.2	10.35	10.03	9.26
NS	1.17	1.12	1.89	0.86	1.77
IN	2.34	2.66	2.34	2.91	2.63
UEW	3.38	3.04	3.02	2.9	2.94
ED	4.4	3.6	3.56	3.63	3.66
IO	3	3.82	3.43	2.8	3.1
EN	2.49	2.52	2.64	2.2	2.46
TYD	2.26	2.22	2.14	1.86	2
TE	1.26	1.32	1.1	1.15	1.02
UAL	6.16	5.34	5.76	4.53	4.26
FAL	8.51	7.26	8.44	6.32	6.49
PAL	7.62	7.66	6.62	7.11	7.25
THL	14.87	14.14	13.96	12.08	12.49
SL	14.33	15.02	14.36	12.96	12.45
FL	13.2	14.28	12.56	11.92	11.65

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