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**Short Communications**

# The First Record of an Hourglass Toad (*Leptophryne borbonica*) in The Core Zone of Bromo Tengger Semeru National Park and Its Ecological Aspects

**Berry Fakhry Hanifa[1]*, Muhamad Aslam Fadhilah[1], Sandra Rafika Devi[2], Muhammad Asmuni Hasyim[1], Luhur Septiadi[3]**

1)Biology Study Program. Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Malang, East Java 65144, Indonesia
2)Faculty of Biology, Universitas Gadjah Mada, D.I. Yogyakarta 55281, Indonesia
3)Wildlife Conservation Society – Indonesia Program, Bogor, West Java 16128, Indonesia
* Corresponding author, email: berryfhanifa@uin-malang.ac.id

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**ABSTRACT**
*Leptophryne borbonica* is an amphibian species known for its sensitivity to environmental changes. Within the expansive Bromo Tengger Semeru National Park (TNBTS), new occurrences of *L. borbonica* have been discovered for the second time in another region of TNBTS (core area in Ireng-Ireng Block, Lumajang). This research provides the newest finding of *L. borbonica* and insights into the habitat assessment and potential food preferences of *L. borbonica* within TNBTS. The methodology involved the examination of morphology and meristics in *L. borbonica.* Habitat assessment and potential food indicated stable values. However, continued vigilance is essential due to vulnerability to volcanic threats.



The Bromo Tengger Semeru Mountains area is a natural preserve that maintains its biodiversity, yet it needs comprehensive data on its animal population, especially amphibians. This circumstance presents a significant opportunity to explore and comprehend the diversity within the TNBTS and its surrounding areas (BBTNBTS 2010). TNBTS holds many mysteries and lacks of data, particularly in the case of reptiles and amphibians (Septiadi et al. 2023a). A species of amphibian was recently discovered as a new distribution record while our team explored the core zone (Blok Ireng-Ireng), namely *Leptophryne borbonica* (Astriyantika et al. 2014).

The *Leptophryne* genus has three species: *L. cruentata*, *L. javanica*, and *L. borbonica. L cruentata* is only distributed in West Java and Central Java (Mumpuni 2001). Hence, it is in the Critically Endangered conservation status (IUCN SSC Amphibian Specialist Group 2019). The bleeding toad is now included in the IUCN Red List as Critically Endangered (Kusrini et al. 2019). At the same time, *L. javanica* was discovered in 2018 and is strictly distributed in West Java and Central Java.

The discovery of new *Leptophryne* populations continued in 2019 when our team found two *L. borbonica* populations in East Java, to be precise in the Coban Cinde and Coban Siuk natural tourism areas, Malang district (Erfanda et al. 2019). This discovery was the latest finding on the distribution of the easternmost *L. borbonica* species on Java Island at that time. Furthermore, we later found a new population in the core area of TNBTS, East Java, which we are still studying. We also found significant differences in morphometrics and webbing formulas in the three East Java populations. From this information, it can be concluded temporarily that the potential presence of *L. borbonica* is still very high, especially on Java Island, and several findings indicate significant morphological differences. Coupled with *L. borbonica* found on the islands of Sumatra and Kalimantan, whose identity remains unclear (Chan & Grismer 2019; Hamidy et al. 2019). Thus, this species must be studied more deeply.

*L. borbonica* (Tschudi, 1838), or the hourglass toad, is a relatively small toad with an hourglass pattern on its dorsal part (Ardiansyah et al. 2014). In research on the distribution of hourglass toads in the East Java region of the Tengger Mountains, the results showed that information related to isolated *L. borbonica* populations is very vulnerable to ecological disturbances, future ecotourism development, infectious diseases, population loss, and local extinction (Erfanda et al. 2019). Therefore, it is fascinating to conduct further research regarding vegetation assessment and potential food preferences in different populations within the same type of mountain.

Research on amphibian diversity has predominantly focused on unveiling cryptic species. At the same time, investigations into other facets of biodiversity, such as habitat assessment and potential food, have progressed slowly and received limited attention (Iskandar 2020). However, it is essential to note that amphibians worldwide encounter a series of direct threats to their long-term survival (Stuart et al. 2008). They are considered more endangered and experiencing more rapid population declines than birds and mammals, necessitating urgent global conservation efforts. Many amphibians in Java inhabit specific micro-habitats, lack reported life histories, are highly sensitive to environmental changes, and are susceptible to local extinction (Cahyadi & Arifin 2019).

Consequently, long-term monitoring is essential to ensure the sustainability of their populations (Storfer 2003). Given the need for more information, conducting a population ecology study focusing on habitat assessment and potential food preferences of *L borbonica* in TNBTS is imperative. Thus, we aim to record the new distribution of *L. borbonica* in the core zone of TNBTS and to provide the habitat assessment and potential food preference.

This research was conducted from February to March 2022 by collecting specimens of *L. borbonica* downstream of the Ireng-Ireng River, located within SPTN Region III of Bromo Tengger Semeru National Park, Senduro District, Lumajang, East Java Province (BBTNBTS 2010) at coordinates 8°06'08"S and 113°04'39"E, has an elevation of 1,200 m.a.s.l (Figure 1).

*L. borbonica* sampling using a virtual encounter survey. A total of 45 individuals, consisting of 36 males and nine females, were collected, recorded, and stored for preservation with alcohol 70% (Matsui 1984). Specimens were selected from one adult male and one adult female (SVL: >20 mm). Morphometric data were measured using digital calipers, following the methods described by Hamidy et al. (2018) and Watters et al. (2016). Measurement results were digitally recorded using the Procreate software.

Data collection related to habitat assessment involved observing vegetation from the Ireng-Ireng River to the bridge (200-300 meters) through purposive sampling survey activities. Exploratory data collection techniques were employed, including direct exploration and documentation methods. The researcher aimed to gather comprehensive information about the plant species at each observation location (Arini & Kinho 2012; Andries et al. 2022).

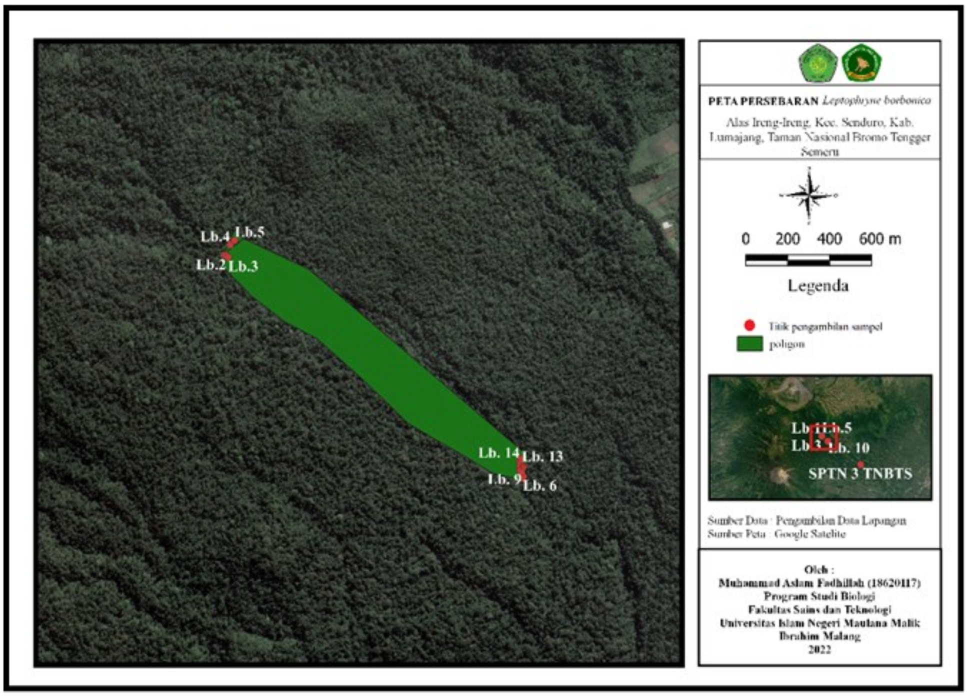


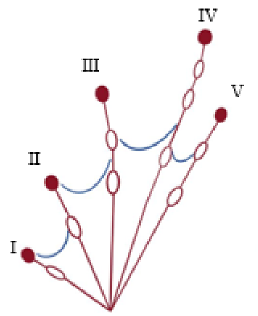
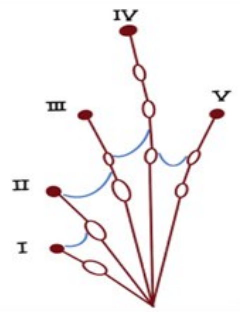
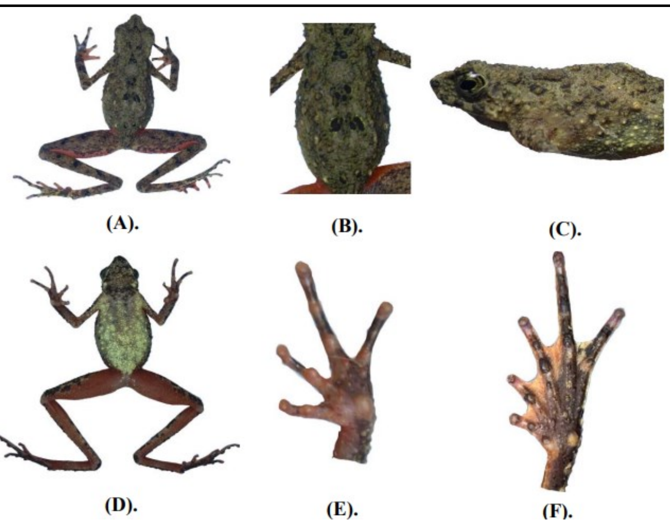

**Figure 1.** Location of the Ireng-Ireng Block (projected by QGIS Desktop 3.36.3).

Data collection regarding potential food was accomplished using insect traps (Pitfall Traps) and hand sorting. Pitfall traps were used to capture ground-surface insects (Southwood & Henderson 2009). Fifteen plots, spaced 10 meters apart and divided into three sets with three repetitions, were used to install Pitfall traps. Subsequently, the collected samples underwent initial hand sorting.

Morphologically, *L. borbonica*) in the Ireng-Ireng, TNBTS exhibits several distinctive characteristics. These include a slender body structure with a Snout-Vent Length (SVL) ranging from 20 to 26 mm in males and 30 to 40 mm in females. The dorsal aspect features a black triangular mark with a rough skin texture and black spots on a light brown body, as depicted in Table 1 (A) and (B). The tympanum is not visible, and the snout protrudes upwards. The orbital region displays a horizontal pupil, while the supra labial region showcases black and white striped motifs and faint parathyroid glands, as shown in Table 1(C). The skin from the cloaca to the supra-orbital region appears rough and wrinkled. The ventral portion is predominantly white, with black markings on the sublabial region (Table 1(D)). The dorsal surface of the femur displays brown colouring with black spots, transitioning to red from the femur to the ventral tibia. The digits of the hand terminate in rounded, lumpy tubercles, as depicted in Table 1 (E), while the metatarsals of the legs appear red. The swimming membrane spans 5 mm on each digit and features black tubercles. Some fresh specimens showed greenish coloration in the dorsum area (Table 1 (F)).

Meristically, *L. borbonica* in the Ireng-Ireng Block of TNBTS exhibits distinct leg webbing patterns (Table 1), which vary between male and female specimens. For male samples from the Ireng-Ireng River population, the membrane formula/webbing formula yielded the following results: (I 0-1 II 0-2½ III 2-2½ IV 3½-1 V) when considering the outer to inner digits. On the other hand, female samples from the Ireng-Ireng River population exhibited the following webbing formula: (I 0-1 II 0-2½ III 2-2½ IV 3½-1 V).

**Table 1.** The morphology of the *L. borbonica* in the Ireng-Ireng, TNBTS.

| Webbing formula | Documentation | Sex |
|---|---|---|
|  |  | Male (I 0-1 II 0-2½ III 2-2½ IV 3½-1 V). |
|  |  | Female (I 0-1 II 0-2½ III 2-2½ IV 3½-1 V). |
| **Sample documentation of *Leptophyne borbonica*** | | |
|  | | A & B. Dorsum<br>C. Lateral view of the head<br>D ventral views<br>E. left hindlimb<br>F. left forelimb |

Based on the information provided above, most morphological characteristics align with the description of *L. borbonica*, as documented by Inger and Stuebing (2005), Erfanda et al. (2019), and Hamidy et al. (2018), hourglass toads are typically tiny, with males having a snout-vent length (SVL) of approximately 23.5 mm and an average SVL of 26.10 mm for the species. They exhibit a slender body habitus characterised by long forelegs and short hind legs, with no discernible bone crest. The snout protrudes slightly above the mouth in profile, and the tip digits are rounded rather than expanded. Parathyroid glands are indistinct, and a distinctive black hourglass mark is on the dorsum. Tympanum and a median singular vocal sac with a vocal slit are evident in males. The skin exhibits a wrinkled texture, covering all body regions, including the forefoot and hindfoot dorsal surface, while the supratympanic fold is notably absent. In terms of coloration, they feature a mottled brown back adorned with black spots on the head, back, and limbs. The groin and ventral surfaces of the fore and hind legs display a reddish hue, with reddish webbing on the back and belly. The abdomen typically exhibits a brownish hue with black and white markings. The chest and throat are blackish, and the limbs bear a distinct black line on the back and a black line on the upper lip. A netted black pattern marks their golden iris. Notably, there is no black triangle marking behind the eye, in contrast to the observations made by Iskandar (1998), who reported black triangles on several specimens from different regions. When compared with Alhadi et al. (2021), differences were found in the snout, which was short and tapered, and the tympanum was oval.

Based on the results of environmental factor measurements during the research (Table 2), the air temperature at the research location ranged from 21.8-24.5°C, humidity levels at 93-94%. Amphibians exhibit a wide temperature tolerance range. They can inhabit temperatures ranging from 0-40°C (Stebbins & Cohen 2021). Meanwhile, species from the same genus, namely *L javanica*, are typically found in environments with temperatures ranging from 15-19.3°C and water temperatures of 16°-18.7°C. Biologically, water temperature affects metabolism, growth, behaviour, interspecific competition, susceptibility to disease, and organism mortality (Coutant 1999). Physically, it can impact the concentration of dissolved gases (Beschta et al. 1987). *L. borbonica*, is not considered an actual aquatic toad and spends most of its life near water bodies, slow-flowing rivers (Iskandar 1998).

**Table 2.** Habitat Parameters of research locations in the Ireng-Ireng, TNBTS.

| Repetition | Physical Parameters | |
|---|---|---|
| | **Temperature** | **Humidity** |
| Upstream | 21.8°C | 93 % |
| Downstream | 24.5°C | 94 % |

A positive correlation exists between the abundance of *L. borbonica* and temperature, albeit weak. Amphibians exhibit specific temperature tolerances and tend to occupy habitats within ranges that support their survival (Freda & Dunson 1986). Anurans, in particular, require higher humidity levels compared to reptiles and other terrestrial animals (Ludwig 1945). Amphibians possess permeable skin that must remain moist, making some anuran species highly dependent on aquatic habitats for survival (Becker et al. 2007; Silva et al. 2014; Hidayah et al. 2018; Devi et al. 2019). It also plays a vital role in anuran communication, thereby contributing to the success of their reproductive processes (Duellman & Trueb 1994; Oseen & Wassersug 2002; Wong et al. 2004).

Besides directly affecting *L. borbonica*, physical factors also influence the vegetation near its habitats. This research also documented the types of vegetation thriving in areas where *L. borbonica* was encountered. It is significant, given that vegetation is crucial in providing shelter for *L. borbonica*, protecting them from predators and UV radiation, and serving as a food source. The protection offered by forest canopy cover also has repercussions for aquatic microhabitats (Paul & Gwynn-Jones 2003). The vegetation identified in this study was riparian vegetation, which typically grows alongside water bodies. Riparian was established that the riverside zone is a refuge for biodiversity and productivity, making it pivotal in land resource and wildlife management. In addition, riverine forest canopies play a crucial role in conserving amphibian diversity, particularly for species specialised in forest habitats (Lemckert 1999; Skelly et al. 2002; Popescu et al. 2012; Provete et al. 2014; Lipinski et al. 2016). Hence, forest vegetation structure is considered a key indicator of biodiversity (Guo et al. 2017), and the present research established a relevant noteworthy correlation.

Vegetation found in the *L. borbonica* encounter area in the Ireng-Ireng, TNBTS (Figure 2), some of the identified vegetation species included: a) *Ageratum conyzoides* b) *Ageratina riparia* c) *Begonia formosana* d) *Dryopteris filix-mas* e) *Homalomena* sp. f) *Impatiens hawkeri* g) *Mallotus barbatus* h) *Mitragyna speciosa* i) *Pilea melastomoides* j) *Plantago major* k) *Schismatoglottis asperata* i) *Selaginella mayeri.* Some *L. borbonica* are often found attached to low-lying leaves near rivers. In contrast, others are located on the soil surface or rocks near watercourses, and some even inhabit the watercourses themselves (Iskandar 1998). *L. borbonica* primarily selects rocks and leaves as substrates for its activities. Many anuran species commonly use substrate as camouflage to deceive predators, preferring those with similar colours to their bodies.


**Figure 2.** Vegetation found in the *L. borbonica* encounter area in the Ireng-Ireng, TNBTS: a) *Ageratum conyzoides* b) *Ageratina riparia* c) *Begonia formosana* d) *Dryopteris filix-mas* e) *Homalomena* sp. f) *Impatiens hawkeri* g) *Mallotus barbatus* h) *Mitragyna speciosa* i) *Pilea melastomoides* j) *Plantago major* k) *Schismatoglottis asperata* i) *Selaginella mayeri.*

*L. borbonica* is not an actual semi-aquatic toad. However, most of these toads are known to spend their entire lives around water areas (Figure 3). This toad is often found around clear, fast-flowing rivers, and indicates soil fertility (Iskandar 1998; Kusrini 2013). It often attached to short herbaceous leaves around rivers; others are found on the surface of soil or rocks around water flows.

The availability of food sources is one factor that determines a species' survival in nature. *Leptophryne*, an insectivorous animal, feeds primarily on small arthropods from the Order Hymenoptera, such as Formicidae and Pheidole (Kusrini et al. 2007). Observation data on *L. borbonica*'s food availability in the TNBTS indicated criteria similar to those obtained using pitfall and hand-sorting methods. In the pitfall method, 491 arthropods were collected (Table 3), resulting in a diversity index of 1.447, a species evenness index of 0.1932 (in the low category), and a medium species richness index of 3.389. Meanwhile, the hand-sorting method yielded 88 types of arthropods, with a diversity index of 2.039, a medium evenness index of 0.5485, and a medium richness index of 2.904.

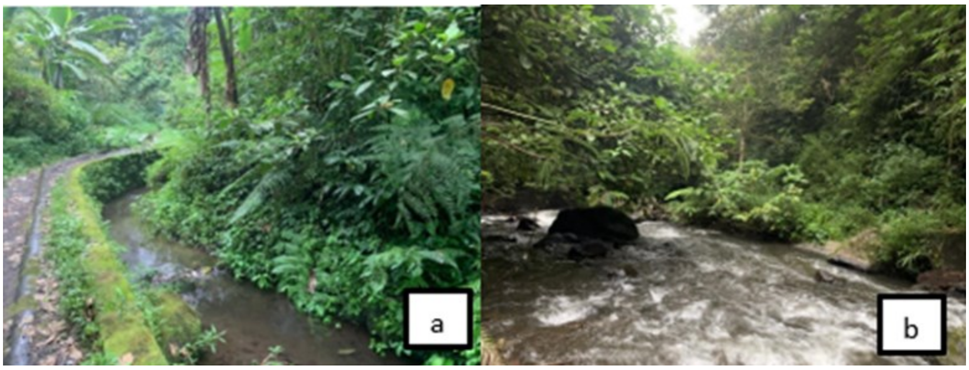



**Figure 3.** Habitat type of *Leptophryne borbonica* in Ireng-Ireng, TNBTS: a) semi-aquatic habitat b) aquatic habitat (Photographed by Muhamad Aslam Fadhillah).

Amphibian diversity is related to environmental conditions because amphibians are very sensitive to environmental changes (Tanjung et al. 2023). Amphibians also play an important role in the food chain, both as predators and prey (Hocking & Babbit 2014; Kanagavel et al. 2017; Carlsson & Tyden 2018; Priambodo et al. 2019). Amphibian responses to the environment are also different for each species. Habitat heterogeneity can influence amphibian richness (Luja et al. 2017). However, habitat heterogeneity can be disturbed due to habitat degradation, natural disasters and anthropogenic activities (Badillo-Saldaña et al. 2016; Berriozabal-Islas et al. 2018). Therefore, monitoring the population must remain a priority (Gillespie et al. 2015)

The greater the diversity, richness, and evenness of ground surface insect species in a given land area, the more stable the forest ecosystem. Dominant orders in the pitfall trap method included Entomobryomorpha (228 individuals), Amphipoda (180 individuals), Orthoptera (27 individuals), and Hymenoptera (27 individuals). In the hand-sorting method, dominant orders included Amphipoda (40 individuals) and Hymenoptera (17 individuals). However, amphibians selectively choose their food based on the available options within their habitat niche. Hence, they can differentiate between different types of prey (Freed 1982). Food preference in amphibians is often linked to their morphology, physiological characteristics, and behaviour (Solé & Rödder 2010). Food availability, particularly from the orders Hymenoptera and Orthoptera, is crucial for maintaining the presence of *L. borbonica* in this niche. Research *on L. cruentata* suggests that their diet predominantly comprises Hymenoptera (ants) at 60.38% and Orthoptera at 6.60% (Freed 1982). It is worth noting that most members of the Hymenoptera order are active explorers, both aerial and terrestrial (Silva et al. 2014; Jaapar et al. 2018).

Among the three *Leptophryne* species in Indonesia (*L. borbonica*, *cruentata*, *L. javanica*), *L. borbonica* is considered the species of least concern by the IUCN because of its wide distribution (Cahyadi & Arifin 2019; IUCN SSC Amphibian Specialist Group 2019). In fact, as a small amphibian inhabiting the Java region, land use changes and deforestation very quickly. This species is also threatened by anthropological and volcanic activity (Erfanda et al. 2019; Septiadi et al. 2023b). The threat of infection by the fungus *Batrachochytrium dendrobatidis* (Bd) can also be a threat to this type because, in West Java, cases of this fungus have been found to have infected several amphibians (Kusrini et al. 2008).

**Table 3**. Arthropod as potential food preference for *L. borbonica* in TNBTS.

| **Index** | **Metode** | |
|---|---|---|
| | **Pitfall** | **Hand Sorted** |
| **Total** | 491 | 88 |
| **Shannon Wiener** | 1.447 | 2.039 |
| **Evenness** | 0.1932 | 0.5485 |
| **Margalef** | 3.389 | 2.904 |
| | **Pitfall Method** | |
| **Ordo** | **Family** | **Total** |
| Araneae | Gnaposidae | 3 |
| | Pholcidae | 1 |
| Entomobryomorpha | Entomobryidae | 228 |
| Amphipoda | Talitridae | 180 |
| Coleoptera | Curculionidae | 2 |
| | Staphylinidae | 3 |
| | Nitidulidae | 1 |
| Orthoptera | Gryllidae | 27 |
| Blattodea | Corydiidae | 1 |
| Poduromorpha | Hypogastruridae | 2 |
| Hemiptera | Miridae | 10 |
| | Cicadellidae | 3 |
| Mesostigmata | Arasitidae | 3 |
| Hymenoptera | Formicidae | 27 |
| **Total** | | **491** |
| | **Hand Sorted Method** | |
| Araneae | Gnaposidae | 7 |
| Orthoptera | Acrididae | 4 |
| Entomobyorpha | Entomobryidae | 3 |
| Amphipoda | Talitridae | 40 |
| Coleoptera | Staphylinidae | 5 |
| | Scarabaeidae | 4 |
| Chilopoda | Scolopendridae | 2 |
| Mesostigmata | Arasitidae | 3 |
| Hemiptera | Coreidae | 3 |
| Hymenoptera | Formicidae | 17 |
| **Total** | | **88** |

## AUTHORS CONTRIBUTION

B.F.H., M.A.H., and L.S. contributed concepts, ideas, research funds, and research equipment, MAF collected data, data preservation, and analysis, and SRD collected data, and wrote the original draft.

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## CONFLICT OF INTEREST

All authors declare no conflict of interests.

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