**Review Article** 

# A REVIEW OF THE BIOLOGY OF *RAFFLESIA*: WHAT DO WE KNOW AND WHAT'S NEXT?

# Review Biologi *Rafflesia*: Apa yang sudah kita ketahui dan bagaimana selanjutnya?

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## Abstrak

Telah dilakukan tinjauan literatur untuk meringkas informasi, terutama karya ilmiah yg baru diterbitkan, pada biologi *Rafflesia*. Sebagian besar publikasi terkini adalah pemberian nama species baru pada *Rafflesia*. Sejak tahun 2002, sepuluh spesies telah ditemukan di Filipina dibandingkan dengan tiga spesies di Indonesia. Karya terbaru filogenetik juga telah dieksplorasi (misalnya sejarah evolusi genus *Rafflesia* dan gigantisme, transfer horizontal gen dan hilangnya genom kloroplas) dan anatomi (misalnya endofit, pengembangan bunga); studi terbaru lainnya berfokus pada biokimia. Sayangnya, masih banyak informasi yang belum diketahui misalnya tentang siklus hidup, biologi dan hubungan ekologi pada *Rafflesia*. Kebanyakan informasi yang tersedia berasal dari hasil pengamatan. Misalnya penurunan populasi telah diketahui secara umum yang kadang kadang dikaitkan dengan kerusakan habitat atau gangguan alam tapi penyebab-penyebab yang lain tidak diketahui dengan pasti. Pertanyaan yang belum terjawab antara lain pada biologi reproduksi, struktur genetik populasi dan keragaman. Dengan adanya perubahan iklim secara global, kita amat membutuhkan studi populasi jangka panjang dalam kaitannya dengan parameter lingkungan untuk membantu konservasi *Rafflesia*.

#### Keywords: Rafflesia, Indonesia, Biologi, konservation, review

### Abstract

A literature review was conducted to summarize information, particularly recently published, on the biology of *Rafflesia*. By far most of the recent papers on *Rafflesia* have named new species. Since 2002, ten species have been discovered in the Philippines compared to three species in Indonesia. Recent work also has explored phylogenetics (e.g. evolutionary history of the genus and of floral gigantism, horizontal transfer of genes and loss of the chloroplast genome) and anatomy (e.g. endophyte, flower development); other recent studies have focused on biochemistry. Unfortunately, we still know very little about the life cycle, biology and ecological relations of *Rafflesia*. Much of what we know comes from observations and not from experimental evidence. Population declines have been noted which are sometimes related to habitat destruction or natural

disturbances but other times the cause(s) are unknown. Questions remain unanswered on its reproductive biology and on its population genetic structure and diversity. With changing climates, we vitally need long-term population studies in relation to environmental parameters.

#### Keywords: Rafflesia, Indonesia, Biology, conservation, review

#### INTRODUCTION

Within the obligate holoparasitic plant family Rafflesiaceae, the genus *Rafflesia* attracts the most attention (Kupicha 1993; Meijer 1993; Nais 2001). This genus is restricted to tropical Southeast Asia – mostly Indonesia, Malaysia, and the Philippines. Its unusual growth habit – with no leaves, stems, or true roots and only a flower seen outside its host – produces the world's largest flower, up to 1 m wide in *R. arnoldii*. Moreover, a single genus of plant – the vine *Tetrastigma* – serves as its host.

Although the biology of *Rafflesia* has always fascinated scientists, there has been renewed interest in the genus over the past few years. New species have been discovered. Studies addressing issues such as the evolution of floral gigantism and endophyte anatomy have been published. All of this interest comes at a time when efforts for the conservation of *Rafflesia* are vitally important, with the pressing issues of climate change and tropical forest destruction.

The purpose of this paper is to review the biology of *Rafflesia*, paying special attention to recent studies. We briefly summarize the literature that has been published on taxonomy, phylogenetics, morphology and anatomy, biochemistry, ecology and conservation biology for species in the genus. To this end, we highlight aspects of the biology of *Rafflesia* that critically need to be addressed and make recommendations for future studies.

#### TAXONOMY

Meijer (1997) provided a comprehensive treatment of *Rafflesia* in Flora Malesiana. He listed 14 species: *R. arnoldii* R. Br. (*R. arnoldii* var. *arnoldii*), *R. atjehensis* Koord. [*R. arnoldii* var. *atjehensis*  (Koord.) Meijer], *R. cantleyi* Solms-Laubach, *R. gadutensis* Meijer, *R. hasseltii* Suringar, *R. keithii* Meijer, *R. kerrii* Meijer, *R. manillana* Teschemaker, *R. micropylora* Meijer, *R. patma* Blume, *R. pricei* Meijer, *R. rochussenii* Teijsm. & Binn., *R. schadenbergiana* Göpp., and *R. tengku-adlinii* Salleh & Latiff.

Rafflesia zollingeriana Koord. was considered a form of *R. patma* by Meijer (1997), even though Koorders described the species in 1919 as distinct. In the treatment, Meijer (1997) also included five "incompletely known species": *R. borneensis* Koord., *R. ciliata* Koord., *R. titan* Jack, *R. tuan-mudae* Becc., and *R. witkampii* Koord. These five species were considered to be incomplete due to the lack of wellpreserved specimens or a convincing type specimen, to a poor description, or to a description based on an immature bud. Nais (2001) re-instated *R. tuanmudae*, *R. witkampii*, and *R. zollingeriana* in his account on *Rafflesia*.

Since Meijer's (1997) treatment, many new species have been described from Indonesia, Malaysia, and the Philippines. Only one species is known from Thailand and one from Brunei (Meijer 1997), with no additional species having been found in these countries. Three species have been discovered in Indonesia, all from Sumatra: *R. bengkuluensis* Susatya, Arianto & Mat-Salleh (Susatya *et al.* 2005); *R. meijeri* Wiriadinata & Sari (Wiriadinata and Sari 2009); and *R. lawangensis* Mat-Saleh, Mahyuni & Susatya (Mat-Salleh *et al.* 2010). With Meijer (1997) and Nais (2001) assessments and the addition of these three new species, the total number of *Rafflesia* species in Indonesia is 14.

In Malaysia, Meijer (1997) recognized six species: *R. keithii, R. pricei*, and *R. tengku-adlinii* found in Sabah and Sarawak and *R. cantleyi, R. hasseltii*, and *R. kerrii* in Peninsular Malaysia. *Rafflesia hasseltii* was regarded as endemic to Sumatra, Indonesia, until 1993 when it was discovered in Peninsular Malaysia (Wong and Latiff 1994). However, it would remain until 2002 for suitable specimens to be collected and studied. Upon further observation, Latiff and Wong (2003) distinguished R. azlanii Latiff & M. Wong with comparisons made to R. cantleyi and R. hasseltii. Due to flower similarity among these three species, they stated "...indeed Meijer in 2001 had hinted that the examples of the new species might be a natural hybrid between R. cantleyi and R. hasseltii." Sofiyanti et al. (2007) examined eleven R. hasseltii flowers from Indonesia and Malaysia and found that the flower diameter, ratio of aperture to diaphragm, and coverage and density of warts (blotches) were very diverse. The most recent discoveries of Rafflesia from Malaysia are R. lima-lidah Wong & Gan (Wong and Gan 2008); R. su-meiae Wong, Nais & Gan (Wong 2009); R. sharifah-hipsahiae Adam, et al. Mohammed, Aizat-Juhari & Wan (Adam et al. 2013); and R. parvimaculata Sofiyanti, Mat-Salleh, Khairil, Zuhailah, Mohd. Ros & Burslem (Sofiyanti et al. 2016), bringing the total of number of species in Malaysia to 12. However, R. lima-lidah has not been formally described.

Barcelona et al.'s (2002) discovery of R. speciosa Barcelona & Fernando from Panay Island was a historic event in the Philippines since the last identified from there Rafflesia was R. schadenbegiana in 1885 (Lays 2006) and until 2002 only two species were known (R. manillana, R. schadenbergiana) (Meijer 1997). The finding of R. speciosa was a turning point for Rafflesia discovery in the Philippines since several new species were recorded afterwards (Barcelona et al. 2009a). Rafflesia manillana was used to describe plants from Luzon and Samar by Meijer (1997), Nais (2001), and Fernando and Ong (2005). However, Madulid and Agoo (2007) re-examined these plants and concluded that the species in Samar was *R. manillana* while that in Luzon was new, R. panchoana Madulid, Buot & Agoo. Later, Pelser et al. (2013) resurrected and neotypified R. lagascae Blanco for the Luzon population named as R. panchoana. Rafflesia schadenbergiana was rediscovered by Lays (2006),

with no collections made, and then by Barcelona et al. (2008a), with a collection of a newly opened flower. Fernando and Ong (2005) revealed R. mira Fernando & Ong (syn. R. magnifica Madulid, Tandang & Agoo) from Mindanao. In 2006, Barcelona et al. reported a new species from southern Luzon naming it R. baletei Barcelona & Cajano and Galang and Madulid found R. lobata Galang & Madulid from Panay Island. In 2007, two separate teams (Barcelona et al. 2007; Madulid et al. 2007) identified R. philippensis Blanco (syn. R. banahawensis Madulid, Villariba & Agoo; R. banahaw Barcelona, Pelser & Cajano) from Luzon. Other new species have been recognized from northern Luzon by Barcelona et al. (2008b, 2009b), R. leonardi Barcelona & Pelser and R. aurantia Barcelona, Co & Balete, and from Mindanao, R. verrucosa Balete, Pelser, Nickrent & Barcelona (Balete et al. 2010) and R. mixta Barcelona, Manting, Arbolonio, Caballero & Pelser (Barcelona et al. 2014). Most notable, Galindon et al. (2016) described R. consueloae, the world's smallest Rafflesia, on Luzon Island. The total number of species in the Philippines is 13.

The total number of *Rafflesia* worldwide is about 36 taxa, with the highest number from Indonesia (14) and a nearly equal number from the Philippines (13) and Malaysia (12). The Philippines is leading the way in the most number of new species being described (11) since 2002. However, a small number of the 36 species are known from very poor specimens (e.g. only a bud), the taxonomic validity of a few species have been questioned, and some species have not been recorded for many years and might be extinct. Clearly, a current assessment of the known distribution and taxonomy of all species, particularly little known species, is urgently needed.

#### PHYLOGENETICS

Based on mitochondrial DNA, recent studies place Rafflesiaceae as a member of Malpighiales (Barkman *et al.* 2004, 2007; Wurdack and Davis 2009). Phylogenetic studies have revealed that floral gigantism in *Rafflesia* was relatively recent and was rapid with floral diameter increasing an average of 20 cm/million years (Davis et al. 2007; Barkman et al. 2008; Davis 2008); that genes in Rafflesia may possibly have been horizontally transferred from the host Tetrastigma (Davis and Wurdack 2004; Molina et al. 2014; Krause 2015); the potential loss of the chloroplast genome (Molina et al. 2014); that the Indonesian, Bornean, Peninsular Malaysian and Philippine species are monophyletic (Bendiksby et al. 2010); and that a dramatic increase in diversification may be explained by Mid-Miocene to Pliocene rainforest experiencing favorable conditions as well selection promoting as natural character displacement in flower size (Bendiksby et al. 2010).

#### **MORPHOLOGY AND ANATOMY**

Many studies have been carried out on the morphology and anatomy of Rafflesia (Justesen 1922; Olah 1960; Meijer 1958; Bouman and Meijer 1994; Bänziger 2004; Sofiyanti et al. 2007; Mursidawati 2012). Recent studies have illuminated fascinating aspects of the flower anatomy as well as the endophyte of *Rafflesia*. Using comparative studies of development and gene-expression patterns, Nikolov et al. (2013) showed that flowers of Rafflesia and Sapria are constructed very differently despite being morphologically very similar and are not homologous. Nikolov et al. (2014a) concluded that the endophyte develops from a proembryo and that the flowering shoot arises from the undifferentiated endophyte. The host does not react to the parasite except when the flower forms. In fact, they also suggested the possibility that the endophyte could be a commensal or mutually beneficial partner to its host. In addition, the surfaces of the floral apex and of the ovarial clefts are exposed secondarily during an extraordinary process of cell separation, which has not been documented in any other angiosperm (Nikolov et al. 2014b).

Bouman and Meijer (1994) suggested that flower diameter and size of ovules were positively related among species. In contrast, Sofiyanti and Yen (2012) found no pattern between these variables for *R. azlanii, R. cantleyi, R. hasseltii,* and *R. kerrii.* In their study, *R. kerrii* which has the largest flower among the four species had a smaller ovule than *R. azlanii* and *R. cantleyi. Rafflesia* seeds are usually chestnut-shaped and brown when fresh and about 500-1500  $\mu$ m in size (Mursidawati 2012; Sofiyanti and Yen 2012).

#### BIOCHEMISTRY

Only over the past eight years has the biochemistry and natural product chemistry of Rafflesia been explored. Plants of R. kerrii have been found to be rich in tannin (Kanchanapoom et al. 2007). Alkaloid (nicotine and caffeine) and phenolic (catechin, proanthocyanidin, phenolic acid) compounds have been detected in R. hasseltii (Sofiyanti et al. 2008; Zulkffle et al. 2014), and an antioxidant compound was detected from R. kerrii flower extract (Puttipan and Okonogi 2014). Extracts from R. hasseltii have been reported to be effective in accelerating wound healing (Abdulla et al. 2009). Furthermore, anti-microbial activity against Candida albicans was detected in an isolate of an endophytic fungus from R. cantleyi (Refaei et al. 2011).

#### ECOLOGY

#### **Buds and flowering**

High mortality rate is common in *Rafflesia* especially for buds that are less than 5 cm diameter and that grow on the ground (Meijer 1984; Hidayati *et al.* 2000; Galang 2007; Susatya 2007). The mortality can be due to natural disasters, human disturbances, high humidity/precipitation, insect infestations, trampling, predation, or resource limitation (Meijer 1985, 1997; Emmons *et al.* 1991; Zuhud *et al.* 1994; Hidayati *et al.* 2000; Nadia *et al.* 2012; Nikolov *et al.* 2014a). Justesen (1922) noted termites inside a dead bud of *R. arnoldii*; however, these buds may have already been dead before termites attacked them since he stated that termites would not attack healthy buds or immature fruits.

Most flowers of *Rafflesia* are found on the ground attached to the vine *Tetrastigma*; however,

sometimes they are observed on a vine up in a tree. For instance, Galang (2007) recorded five flowers of R. lobata at 5 m, two at 7 m, and one at 10 m above the ground. In general, there is no distinct flowering season for Rafflesia. For example, R. arnoldii and R. patma have been observed flowering at any time of the year (Hidayati and Susatya, pers. obs.; Mursidawati et al. 2014). In contrast, flowering appears to be seasonal, coinciding with the hottest and driest time of the year for R. kerri as open flowers have been reported from January to March (Meijer and Elliott 1990). The ratio of male:female flowers in populations is high: 22:8 (27% female flowers) in R. keithii, 89:2 (36% female flowers) in R. pricei, 7:2 (50% female flowers) in R. tengku-adlinii (Nais 2001), 6:1 (14%) in R. Lobata (Galang 2007), and 15:1 (6%) in R. manillana (Yahya et al. 2010). Susatya (pers. comm.) observed seven locations of R. arnoldii for 3 years and found only two female flowers.

#### **Pollination biology**

Typically, more buds than flowers are observed in populations of Rafflesia, wherein flowering is also asynchronous (Justesen 1922; Meijer 1958; Olah 1960; Beaman et al. 1988; Bänziger 1991). Being a dioecious plant, Rafflesia needs pollinators for fertilization to occur. There is strong evidence that in Rafflesia both olfactory and visual cues are necessary to attract and hold pollinators long enough to carry or to transfer the pollen (Beaman et al. 1988). Moreover, Patiño et al. (2002) found that *R. tuan-mudae* is an endothermic flower which plays a role in the mimicry of the flower to attract blowflies. Flies were seen visiting both male and female flowers (Justesen 1922; Meijer 1958; Beaman et al. 1988; Bänziger 1991). In addition, Beaman et al. (1988) stated that the size and color of a Rafflesia flower that contrasts with the environment is an "...adaptation that increases the likelihood that the flower will be discovered by pollinators." Bänziger (1991) mentioned that pollination may occur between remote flowers that are blooming many days or weeks apart since flypollinators can travel a great distance in a few days. However, Nais (2001) found that pollen viability in R.

*kerrii* lasted only 72 hours. Nais (2001; see also Meijer 1997, Bänziger 2004) suggested that *Rafflesia* can produce seeds without fertilization, i.e. agamospermy, which is an issue that needs further study. *Lucilia* and *Chrysomia* were reported to be pollinators for *R. pricei* (Beaman *et al.* 1988), while *Chrysomya*, *Lucilia*, and *Sarcophaga* were found visiting *R. kerrii* (Bänziger 1991, 2004) and *R. patma* (Hidayati *et al.* 2000). Kahono *et al.* (2010) reported an additional five genera of flies visiting a female flower of *R. patma*.

#### Seed dispersal and host infection

The small seeds of Rafflesia are thought to have many possible dispersal agents like wild pigs, ground squirrels (Meijer 1958; Bouman and Meijer 1994; Zuhud et al. 1999; Nais 2001), ants, termites, pangolin (Justesen 1922; Kuijt 1969) and elephants (Kuijt 1969). Emmons et al. (1991) suggested that most likely it is small mammals that are the effective dispersal agents for Rafflesia seeds. They observed adult tree shrew (Tupaia tana) and adult plantain squirrel (Callosciurus notatus) feeding on a fruit of R. keithii. In contrast, Pelser et al. (2013) found numerous ants (Technomyrmex sp., Pheidologeton sp.) inside the fruit wall of R. philippensis. They suggested that ants might be attracted to an elaiosome on the Rafflesia seed and carry seeds away from the fruit to their nest. Here, the seed germinates and infects the root of nearby Tetrastigma. Justesen (1922) believed that infection most likely took place on the underground part of the vine host. Likewise, Bänziger (1991) suspected that soil fauna, mainly arthropods and nematodes, serve as the main agents for new host infection.

#### Life cycle length

Meijer (1997) estimated that the length of the life cycle of *Rafflesia* is 3-4.5 years. More detailed studies have shown that the life cycle length (from seed to seed) for *R. patma* spans ca. 3-4 years (Hidayati *et al.* 2000) and for *R. arnoldii* is 3.5-5 years (Susatya 2007). The development of *R. rochussenii* from visible bud to ripe fruit was ca. 2.5 years (Zuhud *et al.* 1994). Due to this prolonged nature of bud

development and low incidence of flowering study of the life cycle of *Rafflesia* is difficult.

#### **CONSERVATION BIOLOGY**

#### Rarity

Due to its parasitic mode of life and its dependence on a limited number of species of the vine *Tetrastigma* (Chen *et al.* 2011; Zakaria *et al.* 2016), *Rafflesia* is rare and prone to extinction. Other aspects of its biology also leads to its rarity and potential for extirpation: high flower bud mortality, rare pollination events (flowers not opening simultaneously, populations having one sex or having a large sex ratio imbalance), unsuccessful pollination and seed dispersal, long flower development (3-4 years), and highly fragmented populations (Justesen 1922; Meijer 1958; Olah 1960; Hidayati *et al.* 2000; Hikmat 2006; Galang 2007; Yahya *et al.* 2010; Nadia *et al.* 2012).

#### Threats

Populations of some Rafflesia, along with the host, are declining in protected and unprotected forests due to natural disasters, flower collection, logging, and shifting cultivation (Nais and Wilcock 1998; Zuhud et al. 1999; Hikmat 2006; Suwartini et al. 2008; Mursidawati et al. 2014). The main threat for Rafflesia populations is loss of habitat due to logging or to collection of firewood and to conversion of forests into housing or into plantation monocultures of rubber, palm oil, and fruit gardens (Meijer 1985; Hikmat 1988; Ismail 1988; Meijer and Elliot 1990; Bänziger 1991; Nais and Wilcock 1998). In fact, populations of some recently discovered species (e.g. R. lobata, R. manillana, R. philippensis) have been found in severely degraded or fragmented forests (Barcelona et al. 2009a).

Direct human exploitation also is a contributing factor for *Rafflesia* rarity. Due to the conspicuous flowers that attract attention, people often cut them out of curiosity and in doing so also damage the host plants. People also collect buds or flowers because *Rafflesia* is believed to have medicinal properties and is used as a general tonic,

as a cure for fever or back ache, and for childbirth recuperation (Ismail 1988; Meijer and Elliott 1990; Hikmat 2006) or to increase women fertility (Zuhud *et al.* 1999). However, this last practice has not been used for a long time.

#### **Population declines**

Population declines have been noted for several species of *Rafflesia*, but the cause(s) of these declines are sometimes unknown. A site of R. kerri in Khao Sok Nature Reserve, Thailand visited in 1983 was not present in 1989 (Meijer and Elliott 1990). Three sites of R. arnoldii in Taba Penanjung, Bengkulu, Indonesia visited in 2003 did not contain plants in 2014 (Susatya, pers. comm.). Hikmat (2006) reported a rapid decline in populations of R. zollingeriana in East Java, Indonesia. In 1988, he recorded eight sites of this species along the coast of the Indian Ocean in Meru Betiri National Park. By 2003, only three sites contained plants: one site from the original eight and two new sites. Besides habitat destruction caused by a tsunami in 1994, Hikmat (2006) also noted harvesting of flower buds by local people. Suwartini et al. (2008) recorded 256 flower buds from five sites of R. patma in Pangandaran Nature Reserve, West Java, Indonesia in 1989 but only 96 from the same sites in 2007.

#### Ex situ conservation

Attempts to propagate Rafflesia for cultivation are certainly appealing, especially for conservation and for tourism attraction. However, there is little knowledge about how to inoculate host plants and germinate *Rafflesia* seeds. Attempts at germinating seeds in the laboratory or by placing them on living host plants and at tissue culture techniques have not been successful (Nais and Wilcock 1999; Mursidawati et al. 2014; Wicaksono et al. 2016). However, other attempts at inoculation have been successful (see Meijer et al. 1997; Vieldkamp 2007). Rafflesia patma and R. rochussenii growing on a host plant were successfully relocated to Bogor Botanical Garden in 1850, followed by R. arnoldii in 1856 (Meijer 1997; Vieldkamp 2007); recent relocations have not been successful for R. patma (Mursidawati et al. 2014). Recently, using grafting techniques R. patma flowered at the Bogor Botanical Garden (Mursidawati *et al.* 2014; Wicaksono *et al.* 2016). Although pollination was not observed for the grafted plants at Bogor, manual pollination might be tried in the future (Bänziger 2004).

#### In situ conservation

Habitat preservation is an essential part of the conservation of *Rafflesia*. However, many politicians and community leaders believe that conservation actions for Rafflesia are impediments to economic development (Ismail 1988). Collaboration, in mutual trust and respect, between local stakeholders, community leaders, agency heads, and scientists, as well as emphasis on the importance of conservation via social networking and mass media will greatly strengthen initiatives (Lestari et al. 2014). For example, an information network employing village heads and local people was set up in Sabah to report discoveries of new populations of Rafflesia to researchers at the University Kebangsaan Malaysia Sabah Campus. After a few years, at least eleven new sites of Rafflesia were found as compared to three sites known before the network. Many Rafflesia populations occur outside of protected areas or designated parks, and thus, eco-tourism is used in conservation efforts to relieve visitation pressure in protected areas. Ecotourism is a viable option that provides an economic return to landowners (e.g. entrance fees) and does not destroy habitat (e.g. for farming) (Nais and Wilcock 1998; Yulian 2002) as long as it fits within the cultural norms of community access to forest resources (Doolittle 2005). However, establishing ecotourism has to be carefully planned (e.g. from landowner hospitality and travel agency participation, to souvenir manufacturing) and monitored since sites can be over-used to the detriment of Rafflesia (Barcelona et al. 2009a; Lestari et al. 2014).

#### CONCLUSIONS

Progress has been made on the taxonomy, phylogenetics, morphology and anatomy, and biochemistry of *Rafflesia*; however, other aspects of

its biology remain poorly studied. Questions remain unanswered on its reproductive biology (such as agamospermy, seed dispersal and germination, and mode of host inoculation) and on its population genetic structure and diversity. Long-term monitoring of population dynamics as well as data on past and current distributions and status of vitally populations are needed. Preserving populations of Rafflesia and its host plant both outside (with eco-tourism) and inside of parks may become increasingly difficult with climate change. Yet, we know very little about the environmental conditions required for survival, growth, and reproduction of Rafflesia and its host. Efforts to involve local people in conservation strategies need to be continued as well as logistical and funding support for carefully planned ecotourism (e.g. construction of elevated walkways to reduce trampling). An education campaign (in schools and to the general public) on Rafflesia and its host would greatly help in conservation initiatives.

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