Arthropods discovered in lower and upper pitchers of *Nepenthes* at Rampa-Sitahuis Hill, North Sumatra, Indonesia

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Abstract. Tarigan MRM, Corebima AD, Zubaidah S, Rohman F. 2021. Arthropods discovered in lower and upper pitchers of *Nepenthes* at Rampa-Sitahuis Hill, North Sumatra, Indonesia. Biodiversitas 22: 5358-5366. This research aimed to identify the families of arthropods trapped in the upper and lower pitchers of *Nepenthes* in Rampa Sitahuis Hill Area, North Sumatra, Indonesia. This descriptive qualitative research used an experimental method of data analysis. The arthropods were obtained from the upper and lower pitchers of *N. rafflesiana*, *N. gracilis*, *N. eustachya*, *N. ampullaria*, and *N. sumatrana*. Consequently, ten families of arthropods, namely Culicidae, Formicidae, Araneidae, Calliphoridae, Rhyparochromidae, Salticidae, Curculionidae, Blattelidae, Coccinellidae, and Tridactylidae were identified. Culicidae and Formicidae were the most abundant arthropods trapped in *Nepenthes*’ lower and upper pitchers because *Nepenthes* functioned as a microhabitat and breeding ground for Culicidae (adult mosquitoes) and Formicidae (ant larvae). Thus, these species established a mutualistic symbiosis in which ants used the tendrils of *Nepenthes* to lay their eggs and collected nectar generated by the honey glands, while the egg-laying process aids in the pitcher’s breakdown process.

Keywords: Families, mutualism, predation, specimen identification

INTRODUCTION

*Nepenthes* is a carnivorous plant genus found from eastern Madagascar to New Caledonia, Southern China, and several small remote islands in the western Pacific, Malesiana (Yulita and Mansur 2012; Gaume et al. 2016; Nerz and Koch 2018; Rizqiani et al. 2018), as well as Southeast Asia, most notably in Indonesia. Between 2005 and 2014, *Nepenthes* spread throughout Indonesia, with 32 species on Borneo, 29 on Sumatra, ten on Sulawesi, nine on Papua, four on Maluku, and two on Java (Cheek and Jebb 2013; Mansur 2013; Lestari et al. 2018; Rizqiani et al. 2018).

*Nepenthes* is a member of the Nepenthaceae family. Diverse arthropod groups have been observed trapped in their pitchers on numerous occasions (Bauer et al. 2015; Biswal et al. 2018; Hidayat et al. 2018; Rizqiani et al. 2018; Setiawan et al. 2018; Nainggolan et al. 2020). Extrafloral nectarine glands on *Nepenthes* provide a scent that draws arthropods to the pitcher’s lip (Bonhomme et al. 2011; Gorb et al. 2013; Schwallier et al. 2020). In addition, the upper part of the plant contains digestive enzymes referred to as glandular parts that act as digesting sites for trapped prey (Rottloff et al. 2016; Ravee et al. 2018; Saganová et al. 2018; Bekalu et al. 2020; Dkhar et al. 2020; Schwallier et al. 2020).

Although research on arthropods trapped in *Nepenthes* is sparse, Hidayati (2018) discovered that Formicidae and Culicidae were frequently captured in pitchers of *N. reinwardtiana* and *N. mirabilis*, respectively. Additionally, Vong et al. (2021) found Calliphoridae in *N. rafflesiana*, and other research discovered Nitidulidae, Formicidae, Cynipidae, Thomisidae, Culicidae, Chrysomelidae, Flatidae, and Tropiduchidae in *N. gracilis*. Furthermore, arthropods such as Formicidae, Culicidae, Tephritidae, and Culicidae, Formicidae, Liparidae, and Curculionidae were discovered in *N. mirabilis* and *N. rafflesiana*, while legged tadpoles were observed in *N. ampullaria* (Panda and Gunawan 2017). Additionally, Amphipnetidae, Argasidae, Blattellidae, Chaboiodae, Chironomidae, Cosmetidae, Entomobryidae, Euzetidae, and Formicidae, as well as Gryllidae, Linyphiidae, Lycosidae, Lygaeidae, Nabidae, Nicod were found in *N. ampullaria* (Maysarah et al. 2017).

Besides ants, other animals spotted on *Nepenthes* pitchers include flies, mosquitoes, crickets, beetles, frogs, birds, mice (Cheek and Jebb 2013; Lagunday et al. 2017; Lim et al. 2019; Ngai et al. 2020), and *Misumenops nepenthicola* (spider on *N. rafflesiana*) (Karl and Bauer 2020). In addition, *Tupaia montana* and *T. minor* were discovered in pitchers of *N. lowi*, *N. rajah*, *N. macrophylla*, *N. gracilis*, and *N. rafflesiana* (Chin et al. 2010; Greenwood et al. 2011; Pavlovic et al. 2011; Wells et al. 2011; Bauer et al. 2015; van der Ent et al. 2015; Thorogood et al. 2018). Also, *Karivoula hardwickii* was seen in *N. rafflesiana* var. elongata, *N. hemsleyana*, *N. bicalcarata*, and *N. ampullaria* (Grafe et al. 2011; Pavlovic et al. 2011; Schöner et al. 2013; Lim et al. 2014; Thorogood et al. 2018) while *Lepidodactylus cf. lugubris* was found in *N. treubiana* (Nerz and Koch 2018). In addition, sunbirds consume nectar from the peristomes of *N. rafflesiana* and...
N. gracilis (Bauer et al. 2015). Therefore, animals trapped in Nepenthes’ pitchers are attracted to the nectar on its peristome-shaped walls that are strikingly colored due to UV light reflection (Baby et al. 2017; Ngai et al. 2020).

This research was done since discussion regarding the discovery of the arthropod family in Nepenthes’ upper and lower pitchers is still uncommon. It sought to identify the family of arthropods trapped in the upper and lower pitchers of Nepenthes growing in the Rampa-Sitahuis Hill area, North Sumatra Province, Indonesia.

MATERIAL AND METHODS

Research area

This descriptive qualitative research uses exploratory data analysis. The research samples were obtained through direct observations. The research was conducted between January and March 2021. Data collection was carried out at the Rampa-Sitahuis Hill area, North Sumatra Province, Indonesia. The family of arthropods was identified in the Animal Systematics Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatra Utara (USU), Medan, North Sumatra, Indonesia. In the laboratory, the main characteristics of each arthropod specimen were determined.

Research location

Rampa-Sitahuis Hill is located in North Sumatra’s Sitahuis Sub-district, Central Tapanuli District, approximately 15 km from Sibolga City. Rampa-Sitahuis Hill area (01°48’6.51” NL, 98°48’2.19” EL) is located 327 km from Medan’s central business district, with an altitude ranging from 200-800 m above sea level. Rampa-Sitahuis Hill is bounded to the north by Central Tapanuli District, to the south by Sibolga City, to the east by North Tapanuli District, and the west by Tapian Nauli and Kolang Districts. The location features a slope of 0-12% (65.51% of the area), a slope of 12-40% (24.28% of the area), and a slope of 12-40% (8.64% of the area). The air temperature reaches a maximum of 32.8°C and a minimum of 20.9°C. The average air temperature in Sitahuis District is between 21.20 and 26.3°C during the day and lowers to 15-20°C at night. Around the hill, the air humidity is relatively high, ranging between 84.58 and 100%. Additionally, this hill has a hilly and undulating topography, a soil pH of 3-4, and red-yellow podzolic complex rock. The area is approximately 50.52 km². The research location is described in Figure 1.

Figure 1. Map of the Nepenthes locations at Rampa-Sitahuis Hill, Central Tapanuli District, North Sumatra Province, Indonesia
Procedures

The items used in the investigation were plastic bags, labels, alcohol 70%, a camera, and laboratory equipment such as a microscope, tweezers, and a collection container. The plastic bags were used as containers for arthropods found on the lower and upper pitchers of Nepenthes. Alcohol 70% was utilized to preserve the arthropods and keep their bodies intact. The camera was used to snap images of the arthropods as evidence of the research paperwork. Laboratory equipment such as a microscope, tweezers, and collection containers were employed to identify arthropod species found in Nepenthes' upper and lower pitchers. Direct observation at the research site, collection of arthropods from five Nepenthes lower and upper pitchers, labeling of plastic samples, and preserving and recording the quantity and identity of arthropod families were all included in the identification activities. Additionally, arthropods collected from the upper pitchers of Nepenthes were separated from those gathered from lower pitchers. Seventy percent alcohol was added to the samples, and they were labeled. Subsequently, these samples were counted and identified based on Leung et al. (2011) and Nadifah et al. (2017). The data on the number of arthropods were analyzed descriptively to obtain the absolute density, relative density, absolute frequency, relative frequency, and diversity index (Shannon-Wiener index) of the arthropods (Merbach et al. 2007; Giusto et al. 2008; Adlassnig et al. 2011; Gaume et al. 2016).

RESULTS AND DISCUSSION

Nepenthes Species found in the Rampa-Sitahuis Hill

Five species of Nepenthes were discovered in the Rampa-Sitahuis hill area, Central Tapanuli District, North Sumatra Province, Indonesia. Each plant species was classified based on the position of its lower and upper pitchers. Figure 2 shows the location of the five Nepenthes species identified in this study, namely N. rafflesiana, N. gracilis, N. eustachya, N. ampullaria, and N. sumatrana.

Arthropods found in five Nepenthes species

The arthropods discovered intact or damaged in Nepenthes pitchers are listed in Figure 3. Ants (Formicidae) and mosquito larvae (Culicidae) were found in pitchers of N. rafflesiana, N. gracilis, N. eustachya, N. ampullaria, and N. sumatrana. Furthermore, Gryllidae was seen in N. rafflesiana Jack, Calliphoridae in N. sumatrana, and N. eustachya, while Coccinellidae and Curculionidae were observed in N. gracilis and N. eustachya, respectively. In addition, Rhyparochromidae was seen in N. ampullaria and N. sumatrana, Blattellidae in N. ampullaria, Salticidae in N. sumatrana, and Araneidae in N. ampullaria and N. sumatrana.

Each arthropod found in the five Nepenthes species described above varies in size, color, and shape. Arthropods ranging in size from 2 mm to 6 cm were discovered. Each arthropod specimen is unique in color, ranging from black to white, red, yellow, and orange. Some specimens were broad, round, oval, and hairy (Table 1).

Figure 2. Nepenthes species found in the Rampa-Sitahuis Hill, Central Tapanuli District, North Sumatra Province, Indonesia: A. N. rafflesiana (N: 01°48’5.59; E: 0.98°48’2.74; Alt: 355 m asl) (1) lower Pitcher; (2) upper pitcher; B. N. gracilis (N: 01° 48’6.96; E: 0.98° 48’2.04; Alt: 372 m asl) (1) lower pitcher; (2) upper pitcher; C. N. eustachya (N: 01°48’7.01; E: 0.98° 48’2.06; Alt: 370 m asl) (1) lower Pitcher; (2) upper pitcher; D. N. ampullaria (N: 01°48’6.82; E: 0.98° 48’2.27; Alt: 369 m asl) (1) lower pitcher; (2) upper pitcher; E. N. sumatrana (N: 01° 35’7.54; E: 0.98° 53’7.68; Alt: 33 m asl) (1) lower pitcher; (2) upper pitcher
The Araneidae specimen has a body size of 3 mm. Its body color is black and orange. Its black abdomen is rounded and hairy. Its cephalothorax is orange and oval. It has six eyes in a circle and a pair of angled antennae. It also has four pairs of legs with orange and black ends.

Table 1. Arthropod found in Nepenthes lower and upper pitchers

| Arthropods | Description |
|------------|-------------|
| Gryllidae  | The Orthoptera specimen is 4 cm long and black with white bands on the femur. The specimen was identified as a member of the family Gryllidae, typically with a body size of between 2 cm and 6 cm in the imago phase and a body-color range of gray to black. |
| Formicidae | The Hymenoptera specimen measures 0.7 cm in length and has a black thorax-head and a bright red abdomen. The specimen was subsequently assigned to the family Formicidae, which typically has a body length of 0.5 to 3 cm with a color range of black to reddish. |
| Culicidae  | The Culex specimen measures 0.6 cm in length and is all black. This Culex specimen was eventually identified as a member of the family Culicidae, which is typically cosmopolitan in appearance and ranges from 0.5-2 cm, but some are enormous at 4-5 cm in length. |
| Calliphoridae | The Coleoptera specimen measured 0.7 cm in length with an orange body and was found on the plantation’s fringes. The Coleoptera specimen was eventually recognized as a Calliphoridae member, with a body length of 0.5-1cm and a coloration ranging from red to orange with black markings (predominantly a shiny bluish-green). Coleoptera is typically found in forests with a partially closed canopy, where they reside among waste and animal carcasses. |
| Curculionidae | The Curculionidae specimen measures 2-4 mm and has a brick-red to the orange body. Species of this family can be found in trees, such as sago palms, coconuts, and oil palms. |
| Coccinellidae | The Coccinellidae specimen has a wide body shape, oval to nearly round. The head is partially or entirely hidden under the pronotum. The larvae are dark in color; some have reddish-yellow spots and have fork-like spines. They are found on the top of the plant canopy in both wet and dry habitats. |
| Rhyparochromidae | The specimen has a body length of 1-2 cm and a dark brown to black coloration. Species from the family Rhyparochromidae are frequently referred to as dung-colored aphids since these arthropods are ground-dwelling yet fly in search of food. |
| Blattellidae | The Blattellidae specimen has a body size of 9-10 mm and a blackish to brown small oval head. It has a pair of antennae, a pair of elliptical double wings, and a pair of hind wings. |
| Salticidae | Salticidae is a family of invertebrates measured 0.7 cm in length with a vivid body color, a distinct pattern. Species from this family have a brown body and white stripes on the body. Their body is small, and there is a white pattern on its chest area. These arthropods live alone among leaves. They are widespread species that can be found in urban areas and forests. |
| Araneidae | The Araneidae specimen has a body size of 3 mm. Its body color is black and orange. Its black abdomen is rounded and hairy. Its cephalothorax is orange and oval. It has six eyes in a circle and a pair of angled antennae. It also has four pairs of legs with orange and black ends. |

Figure 3. Arthropods trapped in the lower and upper pitchers of Nepenthes: A. Gryllidae (upper pitcher: N. rafflesiana); B. Formicidae (lower pitcher: N. rafflesiana, N. gracilis, N. eustachya, N. ampullaria, N. sumatrana); C. Culicidae (lower pitcher: N. gracilis, N. eustachya and upper pitcher: N. rafflesiana, N. gracilis, N. ampullaria, N. sumatrana); D. Calliphoridae (lower pitcher: N. sumatrana and upper pitcher: N. eustachya, N. sumatrana); E. Coccinellidae (upper pitcher: N. gracilis); F. Curculionidae (upper pitcher: N. eustachya); G. Rhyparochromidae (upper pitcher: N. ampullaria, N. sumatrana); H. Blattellidae (lower pitcher: N. ampullaria); I. Salticidae (upper pitcher: N. sumatrana); J. Araneidae (lower pitcher: N. ampullaria and upper pitcher: N. ampullaria, N. sumatrana)
There are 11 arthropod families found in the lower and upper pitchers of *Nepenthes*. The body of the arthropod that was discovered dead is depicted in Figure 3.

Culicidae species usually live in plants, whereas Formicidae species have a mutualistic symbiosis with *Nepenthes*. All Culicidae specimens were discovered in their larval and live stages. They remained in the pouch fluid, with some demonstrating a larval developmental stage for many days. This finding is consistent with Mogi (2010), Bittleston et al. (2016), and Maysarah et al. (2017), who observed a variety of arthropods in *Nepenthes* in the form of whole individuals, larvae, pupae, and individual body parts like antennae and wings. Based on this, it can be concluded that not all arthropods in *Nepenthes* pitchers are expected to become prey (food), as *Nepenthes* can also serve as a habitat for these invertebrates. Culicidae adults, in particular, employ the pitchers as a mini-bag since they lay eggs in the upper pitchers of *Nepenthes*.

Comparing the number of arthropods in *Nepenthes* pitchers

Table 2 summarizes observations of 386 Arthropods belonging to ten families (133 trapped in the *Nepenthes* lower pitchers and 253 trapped in the *Nepenthes* upper pitchers). Culicidae, Araneidae, and Calliphoridae specimens from the upper pitchers of *Nepenthes* were compared to one another. Formicidae mainly was found in the lower pitchers of *N. eustachya*, *N. ampullaria*, *N. gracilis*, *N. rafflesiana*, and *N. sumatrana*. In contrast, Culicidae specimens mainly were found in the upper pitchers of *N. ampullaria*, *N. gracilis*, *N. rafflesiana*, and *N. sumatrana*.

The research findings suggest that the shape, size, and coloration of upper pitchers of *Nepenthes* are more attractive to arthropods, such as adult Diptera. The decomposers and predators are the Culicidae and Calliphoridae from order Diptera, such as the mosquito and the *Nepenthes* bag fly. Mosquito larvae specifically prey on and destroy the remains of arthropods eaten by *Nepenthes*, while Diptera fly larvae prey on mosquitoes (Scharmann et al. 2013; Hidayati 2018). According to Adlassnig et al. (2011), adult Diptera have high mobility and well-developed senses of sight and smell, which enable them to lay eggs in the upper pitchers of *Nepenthes*.

According to the research findings, arthropod families can be found in both the lower and upper pitchers of *Nepenthes* or the lower or upper pitchers of *Nepenthes*. The arthropods found in *Nepenthes*’s lower and upper pitchers are listed in Table 2.

Members of the Formicidae family coexist alongside *Nepenthes* in a mutualistic symbiotic relationship. Ants lay their eggs inside *Nepenthes* tendrils and aid in the decomposition process in *Nepenthes* pitchers, while *Nepenthes* plants provide nectar released by their honey glands (Bauer et al. 2012). Meanwhile, Araneidae, Rhysarochromidae, Salticidae, Curculionidae, and Coccinellidae species are not attracted to the nectar in the peristome (lip pouch) and lid of the *Nepenthes* bag. According to Adlassnig et al. (2011), the arthropods accidentally slipped into the *Nepenthes* bag since *Nepenthes* preyed on arthropods from these families. Gryllidae and Blattellidae members were discovered in *Nepenthes* pitchers due to the arthropod’s fascination with the UV color trapping mechanism produced by *Nepenthes* pitchers (Baby et al. 2017). This color mechanism results in the lip of the pouch being darker than the body, which turns blue or brilliant green. The color system will attract various arthropod species, including *Acheta domestica*, *Periplaneta americana*, and *Crocothemis servilia* (Takeuchi et al. 2015).

Culicidae and Formicidae families were found in the pitchers of *N. eustachya*, *N. gracilis*, *N. ampullaria*, *N. rafflesiana*, *N. sumatrana*. Meanwhile, Araneidae and Rhysarochromidae were discovered in *N. ampullaria*, and *N. sumatrana* and Calliphoridae in *N. sumatrana* and *N. eustachya*. Salticidae, Curculionidae, Blattellidae, Coccinellidae, and Gryllidae were found in the pitchers of *N. sumatrana*, *N. eustachya*, *N. ampullaria*, *N. gracilis*, *N. rafflesiana*, respectively. In addition, 45 arthropods from the Culicidae family were found in the upper pitcher of *N. gracilis*, while 44, 22, 23, 14, and 10 were found in the upper pitchers of *N. ampullaria*, *N. rafflesiana*, *N. sumatrana* and in the lower pitchers of *N. eustachya* and *N. gracilis*, respectively. In addition, 22, 21, 20, 18, and 12 Formicidae were found in the lower pitchers of *N. eustachya*, *N. ampullaria*, *N. gracilis*, *N. rafflesiana*, and *N. sumatrana*, respectively. Five species of Araneidae were found in the lower pitcher of *N. ampullaria* and three species of Araneidae were found in the upper pitchers of *N. ampullaria* and *N. sumatrana*.

| Families of arthropods | Species of Nepenthes | Number of arthropods |
|-----------------------|----------------------|----------------------|
|                       |                      | Lower pitcher | Upper pitcher |
| Culicidae             | *N. eustachya*       | 14           | -            |
|                       | *N. gracilis*        | 10           | 45           |
|                       | *N. ampullaria*      | -            | 44           |
|                       | *N. rafflesiana*     | -            | 24           |
|                       | *N. sumatrana*       | -            | 23           |
| Formicidae            | *N. eustachya*       | 22           | -            |
|                       | *N. ampullaria*      | 21           | -            |
|                       | *N. gracilis*        | 20           | -            |
|                       | *N. rafflesiana*     | 18           | -            |
|                       | *N. sumatrana*       | 12           | -            |
| Araneidae             | *N. ampullaria*      | 5            | 3            |
|                       | *N. sumatrana*       | 3            | -            |
| Calliphoridae         | *N. sumatrana*       | 3            | 10           |
|                       | *N. eustachya*       | -            | 6            |
|                       | *N. ampullaria*      | -            | 16           |
| Salticidae            | *N. sumatrana*       | -            | 18           |
| Rhysarochromidae      | *N. eustachya*       | -            | 12           |
|                       | *N. ampullaria*      | 8            | -            |
| Curculionidae         | *N. eustachya*       | -            | 6            |
| Blattellidae          | *N. ampullaria*      | -            | 6            |
| Coccinellidae         | *N. gracilis*        | -            | 5            |
| Gryllidae             | *N. rafflesiana*     | -            | 5            |
| Total                 |                      | 133          | 253          |
Ten arthropods from the family Calliphoridae were found in the upper pitcher of *N. sumatrana*, six in *N. eustachya*, and 3 were found in the lower pitcher of *N. sumatrana*. However, arthropods from the family Rhyparochromidae were only found in the upper pitchers of *N. sumatrana* (28) and *N. ampullaria* (16). A total of 18 arachnids from Salticidae were seen in the upper pitcher of *N. eustachya*. In addition, only 8 species from Blattellidae were found in the lower pitcher of *N. ampullaria*. In the upper pitchers of *N. gracilis* and *N. rafflesiana*, six Coccinellidae and five Gryllidae were found (Figure 3).

The absolute density, relative density, absolute frequency, relative frequency of each arthropod found in *Nepenthes* lower and upper pitchers was described.

The highest absolute frequency (FM = 5) and relative frequency (FR = 16.66%) were found in the upper pitcher of *Nepenthes* belonging to the families Cuculidae, Rhyparochromidae, Salticidae, Curculionidae, Calliphoridae, Coccinellidae, Gryllidae, Araneidae, and Formicidae. Besides, the absolute frequency and the relative frequency in the lower pitcher of *Nepenthes* of the families Formicidae, Cuculidae, Blattellidae, Araneidae, Calliphoridae, Rhyparochromidae, Salticidae, Curculionidae, Coccinellidae, Gryllidae are 2 and 12.49%, respectively. These results indicate that the arthropods were dominantly found in the upper pitcher of *Nepenthes*. The absolute density, relative density, absolute frequency, and relative frequency of each arthropod found in the lower and upper pitchers of *Nepenthes* can be seen in Table 3.

The index of arthropod diversity in the lower and upper pitchers of *Nepenthes* is different. The diversity index of arthropods discovered in the lower pitcher of *Nepenthes* (1.86) is lower than the diversity index of arthropods found in the upper pitcher of *Nepenthes* (2.32). This finding demonstrates that a greater number of arthropods were discovered in the upper pitcher of *Nepenthes* than in the lower pouch. The Cuculidae is the most frequently encountered arthropod family in the upper pitcher of *Nepenthes*. Cuculidae prefers the shady and moist upper pitcher of *Nepenthes* because these species have keen senses of sight, smell, and flying, making it easier for them to lay their eggs in the upper pitcher of *Nepenthes* (Adlassnig et al. 2011; Vong et al. 2021). Table 4 shows the diversity index for each arthropod family identified in *Nepenthes'* lower and upper pitchers.

Based on the research findings, Formicidae and Cuculidae accounted for the highest number of arthropods trapped by *Nepenthes* (Rembold et al. 2010; Bittleston et al. 2016; Panda and Gunawan 2017; Hidayati 2018). Arthropods of these families are the main prey for *N. eustachya, N. ampullaria, N. gracilis, N. rafflesiana, and N. sumatrana*. These arthropods are frequently found on land and have a high threshold for sweet tastes. As a result, when they are on the lips of *Nepenthes* pitchers, they receive an indirect indication of nectar’s existence, which attracts them inside the pitchers. Additionally, these arthropods are prone to falling into plants due to their slippery lips (Bonhomme et al. 2011; Buch et al. 2013). The lips of *N. eustachya, N. ampullaria, N. gracilis, N. rafflesiana, and N. sumatrana* pitchers are brighter than the rest of the body, the pitchers’ edges are smooth and jagged, and the nectar is found at the gland’s top (Buch et al. 2015; Chou et al. 2015; Bekalu et al. 2020; Schwaller et al. 2020). *Nepenthes* pitchers also have a distinct odor caused by extrafloral nectarine glands, which draws land arthropods to the lips of their pitchers (Gorb et al. 2013; Schwaller et al. 2020).

**Table 3.** Absolute density, relative density, absolute frequency, the relative frequency of each arthropod found in *Nepenthes* lower and upper pitchers

| Families of arthropods | Species of Nepenthes | Lower pitcher | Upper pitcher |
|------------------------|----------------------|--------------|--------------|
|                        |                      | KM | KR (%) | FM | FR (%) | KM | KR (%) | FM | FR (%) |
| Culicidae              | *N. eustachya*       | 14 | 10.52  | 2  | 12.49  | 0  | 0      | 0  | 0      |
|                        | *N. gracilis*        | 10 | 7.51   | 1  | 6.25   | 45 | 17.78  | 5  | 16.66  |
|                        | *N. ampullaria*      | 0  | 0      | 0  | 0      | 44 | 17.39  | 5  | 16.66  |
|                        | *N. rafflesiana*     | 0  | 0      | 0  | 0      | 24 | 9.48   | 2  | 6.66   |
|                        | *N. sumatrana*       | 0  | 0      | 0  | 0      | 23 | 9.09   | 2  | 6.66   |
| Formicidae             | *N. eustachya*       | 22 | 16.54  | 2  | 12.49  | 0  | 0      | 0  | 0      |
|                        | *N. ampullaria*      | 21 | 15.78  | 2  | 12.49  | 0  | 0      | 0  | 0      |
|                        | *N. gracilis*        | 20 | 15.03  | 2  | 12.49  | 0  | 0      | 0  | 0      |
|                        | *N. rafflesiana*     | 18 | 13.53  | 2  | 12.49  | 0  | 0      | 0  | 0      |
|                        | *N. sumatrana*       | 12 | 9.02   | 2  | 12.49  | 0  | 0      | 0  | 0      |
| Araneidae              | *N. ampullaria*      | 5  | 3.75   | 1  | 6.25   | 3  | 1.18   | 1  | 3.33   |
|                        | *N. sumatrana*       | 0  | 0      | 0  | 0      | 3  | 1.18   | 1  | 3.33   |
| Curculionidae          | *N. eustachya*       | 3  | 2.25   | 1  | 6.25   | 10 | 3.95   | 1  | 3.33   |
| Rhyparochromidae       | *N. sumatrana*       | 0  | 0      | 0  | 0      | 6  | 2.37   | 1  | 3.33   |
|                        | *N. ampullaria*      | 0  | 0      | 0  | 0      | 38 | 15.01  | 4  | 13.33  |
| Salticidae             | *N. sumatrana*       | 0  | 0      | 0  | 0      | 16 | 6.32   | 2  | 6.66   |
| Curculionidae          | *N. eustachya*       | 8  | 6.01   | 1  | 6.25   | 0  | 0      | 0  | 0      |
| Blattellidae           | *N. ampullaria*      | 8  | 6.01   | 1  | 6.25   | 0  | 0      | 0  | 0      |
| Coccinellidae          | *N. gracilis*        | 0  | 0      | 0  | 0      | 6  | 2.37   | 1  | 3.33   |
| Gryllidae              | *N. rafflesiana*     | 0  | 0      | 0  | 0      | 5  | 1.97   | 1  | 3.33   |
| Total                  |                      | 133| 99.94  | 16 | 99.94  | 253| 99.94  | 300| 99.94  |
Arthropods of the family Formicidae were discovered dead in pitchers of *N. eustachya*, *N. ampullaria*, *N. gracilis*, *N. rafflesiana*, and *N. sumatrana*, but their remains were found intact in bag fluid. This liquid is clear, whitish, or hazy in color and has a pH of 5-6. On the other hand, Culicidae were discovered alive and in the larval stage in a yellowish and occasionally black liquid (pH 2.8-4.9). This finding is consistent with Hidayat (2016) research, which indicates that the fluid in the pitcher of *N. ampullaria* is occasionally clear or cloudy and frequently contains dead arthropods. Culicidae larvae found in *Nepenthes* pitchers were still alive. Their organs were intact; whereas Formicidae, Araneidae, Calliphoridae, Rhyparochromidae, Salticidae, Curculionidae, and Coccinellidae were found dead with severely damaged body parts (e.g., only a pair of wing fragments were discovered). Additionally, Blattellidae and Gryllidae were discovered dead, but with all body parts intact.

The extreme condition of the *Nepenthes* pitchers’ fluid renders organisms incapable of survival. This finding corroborates Hidayat (2016) assertion that numerous bacterial species reside in this fluid and participate in the substrate degrading process (Biteau et al. 2013; Chou et al. 2014; Gilbert et al. 2020). The presence of bacteria with a high variety index benefits *Nepenthes* growth by providing additional nutrients. Additionally, the presence of bacteria is influenced by a variety of parameters, including the location and growing conditions of *Nepenthes*, nutrition availability, and the state of the pitchers’ fluid (Chou et al. 2014; Takeuchi et al. 2015; Hidayat 2016; Gilbert et al. 2020). The hue of *Nepenthes* pitchers’ liquid is determined by the particles suspended in it, the remains of Arthropod carcasses, and the chemical enzymes involved in the pitchers’ symbiotic interaction with these animals. Additionally, observations indicate that dead arthropods’ remains add a white tint and a distinct yellowish pigment to *Nepenthes* pitchers’ fluid. *Nepenthes* pitchers’ fluid is frequently brimmed with dead arthropods such as ants, spiders, and beetles and living creatures such as mosquito larvae (Culicidae) (Mithöfer 2011; Hidayat 2016; Yong et al. 2021). This finding corroborates Bazile et al. (2012); Moran et al. (2012); Thornham et al. (2012); and Moran et al. (2013) who reported that various arthropod families could exist in the *Nepenthes* pitchers’ fluid.

In addition to rotting arthropods, the *Nepenthes* pitchers’ fluid contains various species of bacteria (Hidayat 2016). Arthropods and other predatory creatures trapped in *Nepenthes* pitchers accelerate decomposition and boost the plant’s nutritional availability (Takeuchi et al. 2015; Hidayat 2016; Gilbert et al. 2020). Adlassnig et al. (2011) assert that the Arthropod family trapped in *Nepenthes* pitchers affects the characteristics of the *Nepenthes* pitchers’ bacteria. *Nepenthes* pitchers create various chemicals, including phosphate, potassium, and other tiny organic molecules, which aid in decomposing dead Arthropods into *Nepenthes*-friendly substrates. This state will undoubtedly affect the liquid ability to serve as a medium for bacteria to proliferate. In addition, the volume of fluid changes according to the plant size, larger pitchers contain more fluid and arthropods. As a result, large arthropods are typically found in *Nepenthes* most conspicuous pitchers.

Most arthropods captured in *Nepenthes* pitchers come from Culicidae and Formicidae. This finding reveals that *Nepenthes* plants can serve as a habitat for Culicidae and a suitable environment for Formicidae larvae. Arthropods of the families Culicidae and Formicidae form a mutualistic symbiosis with *Nepenthes*. Culicidae (adult mosquitoes) use

### Table 4. The diversity indexes of arthropods found in *Nepenthes* lower and upper pitchers

| Families of arthropods | Species of Nepenthes | Lower pitcher | Upper pitcher |
|------------------------|----------------------|---------------|---------------|
|                        |                      | PI | In PI | H’  | PI | In PI | H’  |
| Culicidae              | *N. eustachya*       | 0.105 | -2.25 | 0.24 | 0 | 0 | 0 |
|                        | *N. gracilis*        | 0.075 | -2.59 | 0.19 | 0.177 | -1.73 | 0.31 |
|                        | *N. ampullaria*      | 0 | 0 | 0 | 0.173 | -1.75 | 0.30 |
|                        | *N. rafflesiana*     | 0 | 0 | 0 | 0.095 | -2.35 | 0.22 |
|                        | *N. sumatrana*       | 0 | 0 | 0 | 0.091 | -2.39 | 0.22 |
| Formicidae             | *N. eustachya*       | 0.165 | -1.79 | 0.29 | 0 | 0 | 0 |
|                        | *N. ampullaria*      | 0.158 | -1.84 | 0.29 | 0 | 0 | 0 |
|                        | *N. gracilis*        | 0.150 | -1.89 | 0.28 | 0 | 0 | 0 |
|                        | *N. rafflesiana*     | 0.135 | -1.99 | 0.27 | 0 | 0 | 0 |
|                        | *N. sumatrana*       | 0.090 | -2.40 | 0.22 | 0 | 0 | 0 |
| Araneidae              | *N. ampullaria*      | 0.037 | -3.29 | 0.12 | 0.012 | 4.42 | 0.05 |
|                        | *N. sumatrana*       | 0 | 0 | 0 | 0.012 | 4.42 | 0.05 |
| Calliphoridae          | *N. sumatrana*       | 0.022 | -3.81 | 0.08 | 0.039 | 3.24 | 0.13 |
|                        | *N. eustachya*       | 0 | 0 | 0 | 0.024 | 3.73 | 0.09 |
| Rhyparochromidae       | *N. sumatrana*       | 0 | 0 | 0 | 0.063 | -2.76 | 0.17 |
| Salticidae             | *N. sumatrana*       | 0 | 0 | 0 | 0.071 | -2.64 | 0.19 |
| Curculionidae          | *N. eustachya*       | 0 | 0 | 0 | 0.047 | -3.05 | 0.14 |
| Blattellidae           | *N. ampullaria*      | 0.060 | -2.81 | 0.17 | 0 | 0 | 0 |
| Coccinellidae          | *N. gracilis*        | 0 | 0 | 0 | 0.024 | -3.73 | 0.09 |
| Gryllidae              | *N. rafflesiana*     | 0 | 0 | 0 | 0.019 | -3.96 | 0.08 |
| Total                  |                      | 0.997 | -24.69 | 1.86 | 0.997 | 42.07 | 2.326 |
the plant pouches as small habitats for egg-laying, and larval growth. Formicidae (ants) lay eggs inside N. tendrilis. Ants also contribute to the acceleration of the decomposition process in Nepenthes pitchers. Araneidae, Rhyparochromidae, Salticidae, Carcujionidae, and Coccinelidae are not attracted to Nepenthes petals and peristomes (pitchers’ lips). Arthropods are trapped in Nepenthes pitchers for various reasons, including that Nepenthes plants occasionally prey on insects belonging to these family groups. Simultaneously, Gryllidae and Blattellidae are present in Nepenthes pitchers due to their close association and Nepenthes’ UV color trapping mechanism, which darkens the lips of the plant’s pitchers while lightening its body.

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REFERENCES

Adlassnig W, Peroutka M, Lendl T. 2011. Traps of carnivorous pitcher plants as a habitat: Composition of the fluid, biodiversity and mutualistic activities. Ann Bot 107 (2): 181-194. DOI: 10.1093/aob/mcq238.

Baby S, Johnson AJ, Zachariaj EH, Hussain AA. 2017. Nepenthes pitchers are CO₂-enriched cavities, emit CO₂ to attract prey. Sci Rep 7 (1): 1-10. DOI: 10.1038/s41598-017-11414-7.

Bauer U, Di Giusto B, Skepper J, Grafe TU, Federle W. 2011. The plant mechanism, which darkens the lips of the plant’s pitchers including Salticidae, Curculionidae, and Coccinellidae, are suitable for microbial growth. Formicidae (ants) lay eggs inside the plant pouches as s

Gilbert KJ, Bittleston LS, Naive MAK, Kiszewski AE, Buenavente PAC, Lohman DJ, NE. 2020. Investigation of an elevational gradient reveals strong differences between bacterial and eukaryotic communities cohabiting Nepenthes hyotelmata. Microb Ecol 90 (2): 334-349. DOI: 10.1007/s00248-020-01503-y.

Gorse EV, Baun MJ, Gorb SN. 2013. Development and regeneration ability of the wax coverage in Nepenthes alata pitchers: A cryo-SEM approach. Sci Rep 3: 1-6. DOI: 10.1038/srep03078.

Hidayat S, Helmanto H, Dodo, Purnomo DW, Supriyatna I. 2018. Habitat of Nepenthes spp. in the area of Sampit Botanic Gardens, Central Kalimantan, Indonesia. Biodiversitas 19 (4): 1258-1265. DOI: 10.1002/ecce3.1920.

Hidayat Y. 2016. Analysis jumlah bakteri cairan tumbuhan N. ampullaria yang terdapat di Hutan Cagar Alam Lembah Harau Sumatera Barat. BioCONCETTA 2 (2): 47-55. DOI: 10.22020/bc.2016.v12.1534. [Indonesian]

Hidayat Y. 2018. Keberadaan Nepenthes spp. Hutan Kerangas Tuing bagi serangga. Media Konservasi 23 (3): 203-209. [Indonesian]

Leung A, Pinder A, Edward D. 2011. Photographic Guide and Keys to the Larvae of Chironomides (Diptera) of South-Western Australia. Department of Environment and Conservation, Western Australia.

Lim RJY, Wong SH, Lam WN, Yeo H, Lam-plua SG, Fashing NJ, Wang WY, Cheong LF, Koh JKH, Neo L, Tan HTW. 2019. Preliminary checklist of the inquiline and prey species of Nepenthes ampullaria pitchers across vegetation types in Singapore. Nat Singap 12: 43-61. DOI: 10.26107/NIIS-2019-0006.
Lim YS, Schöner CR, Schöner MG, Kerth G, Thornham DG, Scharmann M, Grafe TU. 2014. How a pitcher plant facilitates roosting of mutualistic woolly bats. Evol Ecol Res 16 (7): 581-591.

Mansur M. 2013. Tinjauan tentang Nepenthes (Nepenthaceae) di Indonesia. Berita Biologi 12 (1): 1-7. [Indonesian]

Maysarah, Ervizar Am, Z. Hikmat A. 2017. Populasi dan habitat Nepenthes ambulicola Jack. di Cagar Alam Mandor, Kalimantan Barat. Media Konservasi 21 (2): 125-134. DOI: 10.29243/medkon.21.2.125-134. [Indonesian]

Merbach MA, Ziska G, Fiala B, Merbach D, Booth WE, Maschwitz U. 2007. Why a carnivorous plant cooperates with an ant-selective defense against pitchers-destroying weevils in the myrmecophetic pitchers plant Nepenthes bicalcarata Hook F. Ecotropica 13: 45-56.

Mithöfer A. 2011. Carnivorous pitcher plants: Insights in an old topic. Phytochemistry 72: 1678-1682. DOI: 10.1016/j.phytochem.2010.11.024.

Mogi M. 2010. Unusual life history traits of Aedes (Stegomyia) mosquitoes (Diptera: Culicidae) inhabiting Nepenthes pitchers. Ann Entomol Soc Am 103 (4): 618-624. DOI: 10.1603/AN10028.

Moran JA, Clarke C, Gowen BE. 2012. The use of light in prey capture by the tropical pitcher plant Nepenthes aristolochioides. Plant Signal Behav 7 (8): 957-960. DOI: 10.4161/psb.20912.

Moran JA, Gray LK, Clarke C, Chin L. 2013. Capture mechanism in palaeotropical pitcher plants (Nepenthaceae) is constrained by climate. Ann Bot 112 (7): 1279-1291. DOI: 10.1093/aob/mct195.

Nadifah F, Muhajar FN, Arisandi D, Lobo ODM. 2017. Identifikasi larva nyamuk pada tempat penampungan air di Pauhukuan Dero Condong Catur Kabsupaten Siemian. J Kesehatan Masyarakat Andalas 10 (2): 172-178. DOI: 10.24893/jkma.v10i2.203. [Indonesian]

Naingolan L, Gultom T, Silionga M. 2020. Inventory of pitcher plant (Nepenthes sp.) and its existence in North Sumatra, Indonesia. J Phys Conf Ser 1485 (1): 1-8. DOI: 10.1088/1742-6596/1485/1/012013.

Nerz J, Koch A. 2018. Vertebrates as prey of pitcher plants: A new case of a gekko (Lepidodactylus cf. lugubris) found in Nepenthes teubiana (Nepenthaceae) on New Guinea. Russ J Herpetol 25 (2): 147-150. DOI: 10.30906/1026-2296-2018-25.2-147-150.

Ngai LW, Yeo HY, Lim RJ, Hong Wong S, Gek Lam-Phua S, Fashing NJ, Neo L, Wang WY, Fah Cheong LC, Ng PY, Tan HT. 2020. A comparative exploration of the inquiline and prey species of Nepenthes rafflesiana pitchers in contiguous and fragmented habitat patches in Singapore. Raffles Bull Zool 68: 838-858. DOI: 10.26107/RBZ-2020-0939.

Panda A, Gunawan EY. 2017. Komposisi takson tingkat suku serangga yang terperangkap dalam kantong Nepenthes spp. di Taman Nasional Sebangau Kalimantan Tengah. Biotropika 5 (2): 36-43. DOI: 10.21776/ab.biotropika.2017.005.02.1. [Indonesian]

Pavlović A, Slováková E, Šantiček J. 2011. Nutritional benefit from leaf litter utilization in the pitcher plant Nepenthes ampullaria. Plant Cell Environ 34 (11): 1865-1873. DOI: 10.1111/j.1365-3040.2011.02382.x.

Ravee R, Salleh FIM, Goh HH. 2018. Discovery of digestive enzymes in carnivorous plants with focus on proteases. PeerJ 6: 1-22. DOI: 10.7717/peerj.4914.

Rembold K, Fischer E, Wetzl MA, Barthlott W. 2010. Prey composition of the pitcher plant Nepenthes madagascariensis. J Trop Ecol 26 (4): 365-372. DOI: 10.1017/s02664741100012x.

Rizgani S, Ariyanti NS, Sulistijorini. 2018. Diversity of lowland Nepenthes (Pitcher Plants) in Bangka Belitung Islands. IOP Conf Ser Earth Environ 197 (1): 1-8. DOI: 10.1088/1755-1315/197/1/012021.

Rottloff S, Miguel S, Bieteau F, Nisse E, Hammann P, Kuhn L, Chicher J, Bazile V, Gaume L, Mignard B, Hehn A, Bourgaud F. 2016. Proteome analysis of digestive fluids in Nepenthes pitchers. Ann Bot 117 (3): 479-495. DOI: 10.1093/aob/mcw001.

Sagavanov M, Bokor B, Stolárík T, Pavlovíč A. 2018. Regulation of enzyme activities in carnivorous pitcher plants of the genus Nepenthes. Planta 248 (2): 451-464. DOI: 10.1007/s00425-018-2917-7.

Scharmann M, Thornham DG, Grafe TU, Federle W. 2013. A novel type of nutritional ant-plant interaction: Ant partners of carnivorous pitcher plants prevent nutrient export by dipteran pitcher infauna. PLoS One 8 (5): 1-11. DOI: 10.1371/journal.pone.0063556.

Schöner CR, Schöner MG, Kerth G, Grafe TU. 2013. Supply determines demand: Influence of partner quality and quantity on the interactions between bats and pitcher plants. Oecologia 173 (1): 191-202. DOI: 10.1007/s00442-013-2615-x.

Schwaller R, van Wely V, Baak M, Vos R, van Heuven BJ, Smets E, van Vugt RR, Gravendeel B. 2020. Ontogeny and anatomy of the dimorphic pitchers of Nepenthes rafflesiana jack. Plants 9 (1): 1-16. DOI: 10.3390/plants9111603.

Setiawan H, Wardhani KAH, Kamaludin, Hutaogol RR, Afriani R. 2018. The diversity of Nepenthes at the post-mining area in Sintang District, West Kalimantan, Indonesia. Biodiversitas 19 (5): 1820-1827. DOI: 10.13057/bioidv/109532.

Takeuchi Y, Chaffron S, Salicher MM, Shimizu-Inatsugi R, Kobayashi MJ, Diway B, von Mering C, Perntaler J, Shimizu KK. 2015. Bacterial diversity and composition in the fluid of pitcher plants of the genus Nepenthes. Syst Appl Microbiol 38 (5): 330-339. DOI: 10.1038/s41467-015-0066.

Thornham DG, Smith JM, Ulmar GT, Federle W. 2012. Setting the trap: Cleaning behaviour of Camponotus schmitzi ants increases long-term capture efficiency of their pitcher plant host, Nepenthes bicalcarata. J Insect Sci 12: 1-11. DOI: 10.1111/j.1365-2435.2011.01937.x.

Thorogood CJ, Bauer U, Hissljc SJ. 2018. Convergent and divergent evolution in carnivorous pitcher plant traps. New Phytol 217 (3): 1035-1041. DOI: 10.1111/nph.14879.

van der Ent A, Sumail S, Clarke C. 2015. Habitat differentiation of the inquiline and prey species of Nepenthes mirabilis in Songkhla Province, Thailand. Biodiversitas 16 (8): 789-807. DOI: 10.1017/s1258-0304-01468-6.

Vong V, Ali A, Ossan S, Thitiplankanakul S, Anant NN, Pengsakul T. 2021. Larval mosquito (Diptera: Culicidae) abundance in relation with environmental conditions of pitcher plants Nepenthes mirabilis var. mirabilis in Songkhla Province, Thailand. Songklanakarin J Sci Technol 43 (2): 431-438.

Wells K, Lakim MB, Schulz S, Ayasse M. 2011. Pitchers of Nepenthes rajah collect faecal droppings from both diurnal and nocturnal small mammals and emit fruity odour. J Trop Ecol 27 (4): 347-353. DOI: 10.1017/S026647411000162.

Yulita S, Mansur M. 2012. The occurrence of hybrid in Nepenthes hokeristana Lindl. from Central Kalimantan can be detected by RAPD and ISSR markers. Hayati J Biosci 19 (1): 18-24. DOI: 10.4308/hjb.19.1.18.