

A PRELIMINARY SURVEY OF THE TAXONOMIC COMPOSITION OF CONTENTS
OF *NEPENTHES SMILESII* HEMSL. PITCHERS IN
BI DOUP – NUI BA NATIONAL PARK (SOUTH VIETNAM)

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Abstract: We present a taxonomic analysis of contents of *Nepenthes smilesii* Hemsley, 1895 (Nepenthaceae) pitchers collected in Bi Doup – Nui Ba National Park (South Vietnam). 230 specimens of insects, belonging to 7 orders and 17 families, as well as 7 specimens of arachnids (2 orders), 2 specimens of chilopods, 2 specimens of isopods and 1 specimen of oligochaetes were identified. Animal composition from *N. smilesii* pitchers is dominated by flightless and poorly flying invertebrate forms. Small ant species *Technomyrmex yamanei* Bolton, 2007 is a predominant prey. Besides ants, Heteroptera and Blattodea are groups dominating in ration, that distinguishes *N. smilesii* from other *Nepenthes* species, studied before in this context.

Introduction

The genus *Nepenthes* L. (Nepenthaceae) includes about 138 tropical species of carnivorous plants commonly known as pitcher plants (McPherson 2011). Unlike some other carnivorous plants (e.g., Droseraceae), their modified leaves (pitchers) are thought to represent a passive type of traps. The pitchers act as pitfalls: they contain a fluid of the plant's own production, which is used to drown the prey (Lloyd 1942). Despite the passive nature of the traps themselves, the prey spectrum of various *Nepenthes* species is far from random, as the plants apply very different trap strategies (e.g., Bonhomme *et al.* 2011; Moran *et al.* 2012; Chin *et al.* 2014).

The first studies of the diversity of *Nepenthes* prey were attempted as far as the beginning of 20th century (Jensen 1910). It was not until 1991, however, that the first quantitative study on *Nepenthes* prey items was published (Jebb 1991, cited after: Moran 1996). Since then a number of thematic studies were published (for example, Kato *et al.* 1993; Moran 1996; Adam 1997; Giusto *et al.* 2008, Rembold *et al.* 2010; Hosoiishi *et al.* 2012). Most were dealing with the Malesian species of *Nepenthes*.

In this work we present a first quantitative study of the prey spectrum of a *Nepenthes* species from the *N. thorelii* complex, *N. smilesii* (Mey *et al.* 2010; F. Mey, pers. comm.).

Materials & Methods

Studies were conducted in the vicinity of Giang Ly forest station located in the south-east part of Bi Doup – Nui Ba National Park, Lam Dong province, South Vietnam. Tropical mid-mountain polydominant forests and mossy forests are predominant vegetation type here. In the locality under study *Nepenthes smilesii* is rare and was found as one population with a small number of individuals (<10 on the area 5×5 m). *Nepenthes* pitchers were collected for analysis on December 24, 2012 in thinned pine forest with predominance of *Pinus kesiya* Royle ex Gordon. The patch of forest is situated on the top of a hill, on a flat plot. Similar forest fragments with predominance of *P. kesiya* can be found in the near vicinity. All pitchers were located at one level, near ground surface.

Description of community including *Nepenthes smilesii* was made on January 07, 2013 by A.N. Demidova and N.G. Prilepsky. Density (in parts of unity) was noted for tree layer, undergrowth, and shrub layer, and total projective coverage (in percentage) was marked for suffruticose-herb layer. Pavlovskij–Braun-Blanquet visual scale of cover-abundance was used for estimation of abundance of trees, shrubs and herbs: [+] – species is poorly present (projective coverage (PC) up to 1% of the description area); [1] – considerable amount (PC forms 1-5% of area); [2] – species is abundant, but PC is lower than 25%; [3] – species is very abundant (PC is 25-50%); [4] – PC is 50-75%; [5] – complete dense cover (PC is 75-100%). Latin plant names are given according to: <http://tropicos.org>.

Coordinates of locality of material collection: 12°11'00.5"N, 108°40'45.4"E, H = 1411 m.a.s.l. Vietnam, Lam Dong province, Bi Doup mountain massif, slope near Giang Ly forest station, pine (*Pinus kesiya*) grass forest, *Nepenthes* population. Community name: pine bamboo-grass forest. Description area: 10×10 m. Density – 0.2, height of the main canopy (*Pinus kesiya*) – 12 m, diameter of tree trunks – 40-50 cm. Degree of species projective coverage is listed in brackets. Undergrowth: *Pinus kesiya*, 0.4-1 m high (1); *Schima* sp., 0.5 m high (+). Shrub layer: *Melastoma* sp., 1.5 m high (1). Total projective coverage of herb layer on sample plot: 70-80%, near *Nepenthes* population (5×5 m plot) – 40%. Herbage composition: *Poaceae* – 3 (4); *Bambusoideae* – 3; *Lycopodiella cernua* (L.) Pic. Serm. – 1-2; *Gleicheniaceae* – 1; *Nepenthes smilesii* Hemsl. – +; *Rubus* sp. – +; *Dianella* sp. – +; *Lindernia* sp. – +. On ground – thin layer of pine needles, branchlets, grass litter. Near sample area – cut down pine trees, fern thickets (Figs. 1-2).

Collected herbarium specimen of *Nepenthes* (Fig. 3) is stored in Herbarium of Moscow University (MW). Copy of herbarium label: “B17: 12°11'00.5"N, 108°40'45.4"E, H = 1411 m.a.s.l. Vietnam, Lam Dong province, Bi Doup mountain massif, to the north of Giang Ly forest station, slope, pine forest (*Pinus kesiya* Royle ex Gordon), near *Fokienia hodginsii* planting. On ground. Leg.: A.N. Kuznetsov, A.N. Demidova, N.G. Prilepsky. Det. genus: A.N. Kuznetsov, S.P. Kuznetsova, A.N. Demidova, N.G. Prilepsky. Det. species: M.S. Nuraliev. *Nepenthes anamensis* Macfarl., 1908. December 24, 2012. On 3 sheets. No. 445”. Subsequently the plant was identified as *N. smilesii* by F. Mey (F. Mey, pers. comm.).

Two fully developed pitchers 15.5 and 10.5 cm long were chosen for the study (Fig. 4). There were a certain variation in size, shape and color of the pitchers; nevertheless, since the plant was trailing without any support, all the pitchers were located at one level near the ground. Pitchers were repeatedly rinsed with alcohol and their contents were analyzed with MBS-10 (LOMO-Eltem, St. Petersburg) with 8× ocular (8×–56× magnification) and Stemi 2000C Carl Zeiss (10×–60×) stereomicroscopes. Photographs of the specimens, except ants, were made by Canon EOS 1000D camera connected to MBS-10 microscope through MFU photo adapter (LOMO-Eltem). Axiocam 105 color camera was used with Stemi 2000C stereomicroscope for ant imaging. Photos were processed in



Figure 1: General view of natural community, in which *Nepenthes smilesii* was found in Bi Doup – Nui Ba National Park (South Vietnam). Photo by Anna Demidova.



Figure 2: Character of herb layer near *Nepenthes smilesii* population. Photo by Anna Demidova.



Figure 3: Herbarium specimen collecting. Anna Demidova in photo. Photo by Nikolay Prilepsky.

Helicon Focus 5.3 (z-stack) and Adobe Photoshop CS5 programs.

Results

242 specimens of invertebrates (without taking into account extremely small, unidentifiable fragments) were observed in the studied samples: 230 specimens of insects from 7 orders and 23 families, as well as 7 specimens of arachnids (2 orders), 2 specimens of chilopods, 2 specimens of isopods and 1 specimen of oligochaetes. Results of identification are listed in Table 1.

The majority of collected insects can be identified up to the family level. Representatives of Dermaptera and Auchenorrhyncha orders (single specimen of each taxon) as well as some Coleoptera and Diptera are present in the samples only as isolated body or wing fragments, so their identification was complicated.

Cockroaches, Blattodea (6.4%): Blattidae – 1 genus, 1 species; Ectobiidae – 2 genera, 3 species. Inhabitants of lower forest layer.

Orthopterans, Orthoptera (1.2%): Gryllacrididae – 1 specimen; Trigoniidiidae – 2 specimens.

Heteroptera (8.9%): Lygaeidae – *Dieuches pamela* Eyles 1973 (5 males, 12 females, 2 larvae); Nabidae – *Stenonabis* sp. (2 females).

Coleoptera (1.7%): two elytra, belonging to the representatives of two families – Lycidae and Chrysomelidae (Fig. 5), others remain unidentified up to the family level (only hind wings are present). Small species.

Parasitic hymenopterans, Hymenoptera, part. Parasitica (1.7%): Charipidae, Chalcidoidea, Diapriidae, Ichneumonidae. (Fig. 6). Represented by very few specimens.

Hymenoptera: Formicidae (68.1%). Predominant group of insects in prey of these samples of *Nepenthes smilesii*. Seven species from 7 genera and 5 subfamilies were found (Table 2).

The dominant species is a background species for the study area from Dolichoderinae, *Technomyrmex yamanei* Bolton, 2007 (Fig. 7, image 1). Quite numerous colonies of this species inhabit



Figure 4: *Nepenthes smilesii* pitchers in nature (left & center). The two pitchers on the right were used for study in the laboratory. Photos by Anna Demidova.

Table 1. Taxonomic composition and abundance of invertebrates found in *Nepenthes smilesii* pitchers in Bi Doup – Nui Ba National Park in South Vietnam.

Class	Order, suborder, superfamily	Family	No. of specimens	%
Hexapoda (insects)	Blattodea:	Blattidae:	1	6.4
		Ectobiidae:	14	
	Dermaptera:		1	0.4
	Orthoptera:	Gryllacrididae:	1	1.2
		Trigonidiidae:	2	
	Hemiptera, Auchenorrhyncha:		1	0.4
	Hemiptera, Heteroptera:	Lygaeidae:	19	8.9
		Nabidae:	2	
		Others:	2	
	Coleoptera:	Lycidae:	1	1.7
		Chrysomelidae:	1	
		Others:	2	
	Hymenoptera:			
	Superfamily Cynipoidea:	Charipidae:	1	1.7
	Superfamily Chalcidoidea:		1	
	Superfamily Proctotrupeoidea:	Diapriidae:	1	
	Superfamily Ichneumonoidea:	Ichneumonidae:	1	
	Superfamily Formicoidea:	Formicidae:	165	
	Diptera:			5.9
	Suborder Nematocera:	Sciaridae:	8	
Suborder Nematocera:	Mycetophilidae:	1		
Suborder Nematocera:	Simuliidae:	1		
Suborder Brachycera:	Phoridae:	2		
Suborder Brachycera:	Tephritidae:	1		
	Others:	1		
Arachnida (arachnids)	Phalangida:		3	1.2
	Acarina:			1.7
	Suborder Oribatida:		1	
	Suborder Thrombidiformes:		2	
	Others:		1	
Chilopoda (chilopods)	Lithobiomorpha:		2	0.8
Eucrustacea (crustaceans)	Isopoda:		2	0.8
Annelida (annelids)			1	0.4
Total:			242	100

Scale = 1 mm



Figure 5: Elytra of beetles: Lycidae family (left), Chrysomelidae family (right). Identification by I.A. Zabaluev. Photo by Evgeny Shcherbakov.

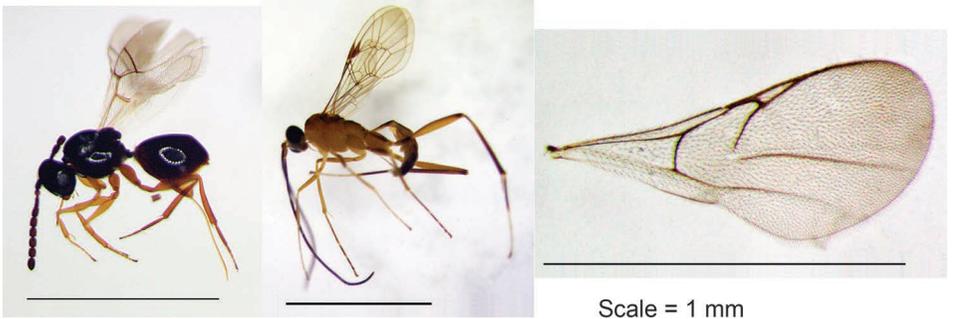


Figure 6: Parasitic hymenopterans: Charipidae family (left), Ichneumonidae family [Cryptinae subfamily] (center), Wing of Diapriidae family (right). Identification by A.V. Antropov. Photo by Evgeny Shcherbakov.

dotted trees and dead wood in lower forest layer, using bark desquamations and various hollows (V.A. Zryanin, personal observation). Three other ant species from pitchers, *Crematogaster* sp., *Tetramorium* cf. *nipponense* Wheeler, 1928, *Polyrhachis halidayi* Emery, 1889 (Fig. 7, images 2-4), also belong to dendrobiont or dendrophil ecological groups. Another three, *Brachyponera* cf. *chinensis* Emery, 1895, *Cerapachys sulcinodis* Emery, 1889, and *Nylanderia* sp. 2 (Fig. 7, images 5-7) may be treated as herpetobionts (*C. sulcinodis* is specialized in raiding the nests of other ants for prey), but in the study area they also prefer to inhabit wood remains and often go up to the herb layer, which is likely the reason they were captured by the *Nepenthes smilesii* pitchers.

Table 2. Species composition and abundance of ants found in *Nepenthes smilesii* pitchers in Bi Doup – Nui Ba National Park in South Vietnam.

No.	Species	Subfamily	No. of specimens
1	<i>Polyrhachis halidayi</i>	Formicinae	4
2	<i>Nylanderia</i> sp. 2*	Formicinae	1
3	<i>Cerapachys sulcinodis</i>	Dorylinae	2
4	<i>Technomyrmex yamanei</i>	Dolychoderinae	129
5	<i>Crematogaster</i> sp. 3*	Myrmicinae	13
6	<i>Tetramorium</i> cf. <i>nipponense</i> (= <i>Tetramorium</i> sp. A)*	Myrmicinae	6
7	<i>Brachyponera</i> cf. <i>chinensis</i>	Ponerinae	10
Total:			165

*These species are readily identifiable by morphological characters on the level of local ant fauna and included in the recently published review (Zryanin, 2013) under indicated codes.



Figure 7: Ants: 1) *Technomyrmex yamanei*, 2) *Crematogaster* sp. 3, 3) *Tetramorium* cf. *nipponense*, 4) *Polyrhachis halidayi*, 5) *Brachyponera* cf. *chinensis*, 6) *Cerapachys sulcinodis*, 7) *Nylanderia* sp. 2. Scale = 1 mm. Identification and photo by Vladimir Zryanin.

Dipterans, Diptera (5.9%). Nematocera: Sciaridae, Mycetophilidae, Simuliidae; Brachycera: Phoridae, Tephritidae (Fig. 8). Mainly small, poorly flying forms.

Among mites (Acarina, 1.7%) there are at least one representative of oribatids (Oribatida) and two representatives of Thrombidiformes (Fig. 9).

Results of quantitative analysis are presented as a diagram (Fig. 10).

Discussion

Results of the taxonomic analysis of the pitcher contents, the first of its kind for *N. smilesii*, show that its nutrition is formed by a wide spectrum of invertebrates, the majority of which is inhabitants of soil surface. Only 9.4% of specimens can be referred to actively flying fauna.

As in case of majority of other *Nepenthes* species (Kato *et al.* 1993; Moran 1996; Adam 1997; Rembold *et al.* 2010; Hosoishi *et al.* 2012), ants form main constituent element of the prey. Almost

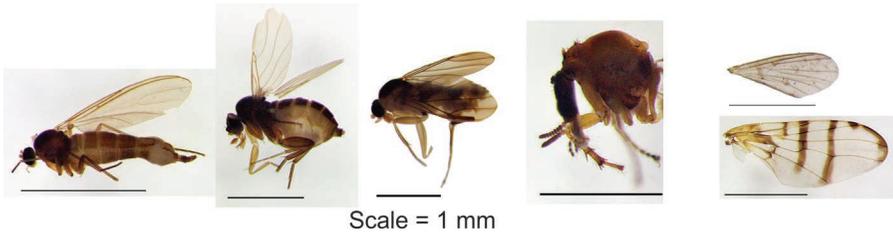


Figure 8: Dipteran (left to right): Sciariidae, Phoridae (2), head and thorax of a black fly Simuliidae, and wings of a fungus gnat Mycetophidae (above) and fruit fly Tephritidae (below). Identification by A.L. Ozerov. Photo by Evgeny Shcherbakov.



Figure 9: Mites: soil oribatid Oribatida (left), Trombidiformes (right). Identification by G.S. Eremkin and E.O. Shcherbakov. Photo by Evgeny Shcherbakov.

all ant species from pitchers are abundant in the study area (Zryanin 2013), however, *Technomyrmex yamanei* obviously predominates in the prey. This is a comparatively small species with sufficiently soft cuticula, which, in turn, can make it an appropriate prey for *N. smilesii*. At the same time, in *N. bokorensis* Mey growing in Cambodia, belonging together with *N. smilesii* to the *N. thorelii* complex, species composition of ants is characterized by the predominance (40% of all ants in the pitchers) of a relatively large *Polyrhachis (Myrma)* species (Hosoishi *et al.* 2012). Predominance of certain species of

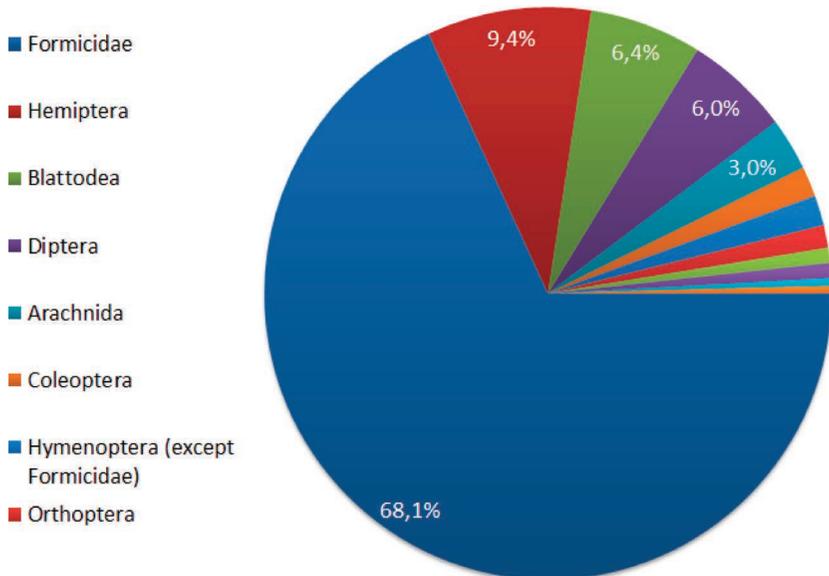


Figure 10: Quantitative composition of representatives of different invertebrate taxa in *Nepenthes smilesii* prey spectrum.

ants in pitchers was also observed in *N. rafflesiana* growing on Borneo. In the latter case, a mechanism of periodical activation and deactivation of trapping activity was described, that allows for capturing of large parties of such prey (Bauer *et al.* 2015). Probably, such mechanism exists in *N. thorelii* complex as well.

We also have myrmecological material taken from several pitchers of *Nepenthes mirabilis* (Lour.) Rafarin, 1869 in June–July 2008 in Binh Chau nature reserve (Ba Ria – Vung Tau province, Vietnam) (unpublished observations). This is the most widespread *Nepenthes* species in lowland Vietnam forests. A total of 17 species of ants from 11 genera, 5 subfamilies (identification by V.A. Zryanin) were found. Ants of four species (*Anoplolepis gracilipes* Smith, 1857, *Pheidole* cf. *plani-dorsum* Eguchi, 2001, *Ochetellus glaber* Mayr, 1862, and *Crematogaster* sp.) made from 40 to 95% of all prey of *N. mirabilis* (A.V. Tiunov, pers. comm.), i.e., on the average, the same as all ants in our study. Nevertheless, no predominance of any single species was observed.

In relation to the other (non-Formicidae) dominating groups of prey, *N. smilesii* differs strongly from other species. Second group (as far as abundance is concerned) is formed by true bugs (Heteroptera), which very rarely get into pitchers of other species with the exception of *N. lowii* Hook., where they make 8.3% of prey (Adam 1997). Moreover, the main mass is formed by only one species, *Dieuches pamela* Eyles, 1973, represented by different age groups.

Finally, 6.4% of prey is formed by cockroaches (Blattodea). Apparently, cockroaches fall into *N. smilesii* pitchers on a regular basis, which is indicated by the presence of their remains of different degrees of decay. Among other species of *Nepenthes*, cockroaches were registered in small amount for *N. × kinabaluensis* and *N. villosa* (per 3%; Adam 1997), though, as an accidental element, were singled out in other species as well (Adam 1997; Giusto *et al.* 2008). Orthopterans inhabiting the same conditions are rarer prey; maybe, as these insects have strong hind legs and are able to move by jumping, they have a certain chance to get out of the trap. On the other hand, the inner slippery surface of the pitchers may hamper the escape. Thus the reasons for the rarity of Orthoptera remain obscure at present.

Other groups (such as Coleoptera and Diptera) are notable in their relative scarcity on *N. smilesii* pitchers compared to those of other lowland species studied to date. Total absence of termites is especially noteworthy, however this may be due to their absence in this particular *Nepenthes* habitat.

We stress that the results presented here are very preliminary. Several factors may have influenced our understanding of *N. smilesii* prey spectrum as evidenced by our sample. Among the biggest are the sample size and the polymorphism of the pitchers. The first is due to the very small size of the *N. smilesii* population in the area studied, where there wasn't much possibility to collect more material. As for the possible pitcher dimorphism, we don't consider it a big factor since all the pitchers were located at one level near the ground, in the same microenvironment and in the presence of the same entomofauna. In turn, local entomofauna and its dynamics obviously influence the pitcher contents, and their contribution are notoriously difficult to study, requiring special long-term collecting efforts. Such efforts are not known for the area inside Bi Doup – Nui Ba National Park, where *N. smilesii* was studied (perhaps excluding ants). The differences in prey spectrum between *N. smilesii* and other species, hinted by our results, make perspective of future studies of all these factors very interesting.

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