

---

# Commensals of *Nepenthes* pitchers

Leonora S. Bittleston

---

## 23.1 Introduction

Carnivorous pitcher plants are elegant systems for biological study. The aquatic pool inside of a pitcher represents a small, relatively self-contained ecosystem. In these pools, pitchers host complex communities of arthropods, fungi, protozoa, and bacteria (“inquilines:” organisms commonly found living within another’s space [e.g., nest, burrow, or pitcher]). Many inquilines prosper within pitcher pools, despite the fact that the pitchers trap, drown, and digest other creatures. But pitchers are not just passive housing—they are parts of living plants, and their internal conditions change over time. To thrive in *Nepenthes* pitchers, inquilines have to contend with acidic conditions, free radicals, viscous fluids, plant chemicals, and other hungry inhabitants. It is still unclear if and how adaptations are necessary for living in pitchers, and also if, how, and to what extent pitcher inquilines influence their host plants. Researchers are just beginning to uncover information about the full diversity of pitcher communities, and which environmental and host characteristics most strongly affect inquiline colonization and persistence.

With over one hundred species ranging from Madagascar to Australia, *Nepenthes* pitcher plants are widespread and have diverse strategies for acquiring nutrients (Chapters 5, 17, 26). Pitchers generally attract insect prey with extrafloral nectar, volatiles, and ultraviolet patterning (Moran et al. 1999, Merbach et al. 2001, Di Giusto et al. 2008, Kurup et al. 2013; Chapters 12, 15). The slippery peristome lip combined with a slippery, often waxy, region inside the top third of the pitcher cause prey to fall in and be unable to escape (Bohn and Federle 2004, Gorb et al. 2005; Chapter 15). The aquatic pool

within a pitcher is a mix of rainwater and secretions from the plant. *Nepenthes* plants can produce their own digestive enzymes, including proteases and chitinases, to break down compounds in insect bodies (Tökés et al. 1974, Eilenberg et al. 2006, Hatano and Hamada 2008; Chapter 16). Autolytic enzymes from drowned prey and free radicals also contribute to the process, and it is likely that microbial enzymes play a role as well (Juniper et al. 1989, Kitching 2000, Chia et al. 2004; Chapter 16). *Nepenthes* pitchers contain some secreted liquid even before opening (Bauer et al. 2009). Upon opening, pitchers are colonized by insects, mites, bacteria, fungi, and protozoa (Kitching 2000, Bauer et al. 2009), and an aquatic food web rapidly forms. Many inquilines living in *Nepenthes* are found only in pitcher habitats, and do not complete their life cycles in other habitats (Beaver 1983).

In this chapter, I briefly review *Nepenthes* pitcher plants as habitats and their common inquilines. I then describe in more detail the recent literature on *Nepenthes* microbial communities, and patterns across pitcher systems. The last sections compare *Nepenthes* pitcher communities to those of surrounding habitats and other pitcher genera, and discuss future directions in the study of pitcher microecosystems. I illuminate what makes pitchers unique as habitats, and hypothesize how pitchers might influence the assembly of their contained communities.

## 23.2 History of *Nepenthes* inquiline studies

Rumphius (1750, in Beekmann 2004) may have been the first to report—in the 1600s—the presence

of living organisms in *Nepenthes* pitchers, but research on these inquilines began in earnest in the early 1900s. The Dutch entomologist Johannes de Meijere published extensive descriptions of his observations of *Nepenthes* inquilines in Singapore (in Part I of Jensen 1910), and proposed a new genus, *Nepenthosyrphus*, for three syrphid flies whose larvae live in *Nepenthes* pitchers. Günther (1913) wrote about the insects of Sri Lanka *Nepenthes*; Dover et al. (1928), working in Singapore, completed what was likely the first comprehensive study of *Nepenthes* pitchers as habitat; and Thienemann (1932) coined three terms: nepenthebiont—specialist inhabitants of *Nepenthes* pitchers; nepenthephils—common inhabitants that also live elsewhere; and nepenthexenes—accidental inhabitants that do not establish persistent populations.

Since Thienemann's time, researchers have studied *Nepenthes* food webs in multiple species and regions. For example, food webs tend to be more complex in regions closer to the center of *Nepenthes* diversity, where more *Nepenthes* species coexist on the same landmass (Beaver 1985, Clarke and Kitching 1993, Clarke 1998, Kitching 2000). Differences among *Nepenthes* species and their physical shapes appear to influence the inquilines that colonize them (Clarke and Kitching 1993, Clarke 1998), and even different forms of the same *Nepenthes* species

can have different food-web structures (Ratsirarson and Silander 1996). There are observable successional patterns within pitchers: Sota et al. (1998) documented how abundances of different inquiline species changed with pitcher age, and how bacterial density increased as pitchers aged and then decreased as they senesced. Bacteria and other microbial organisms generally have been included in depictions of *Nepenthes* food webs as one combined node, because of difficulties in identifying microbial species and their functional roles.

Recent progress in understanding associations among *Nepenthes* pitchers and other organisms has gone in two directions: the very small and the very unusual. Detailed data on bacterial communities have only emerged in recent literature (Chou et al. 2014, Takeuchi et al. 2015, Sickel et al. 2016, Bittleston 2016, Chan et al. 2016, Kanokratana et al. 2016) but the (even smaller) viruses living within *Nepenthes* pitchers have not yet been characterized, and roles of viruses in assembly of inquiline communities are completely unknown. In unusual cases, *Nepenthes* species have been found to form mutualistic associations with ants, bats, and tree shrews (Clarke and Kitching 1995, Clarke et al. 2009, Grafe et al. 2011, Bazile et al. 2012, Scharmann et al. 2013; Chapters 15, 26). Inquilines recorded to date from *Nepenthes* pitchers are listed in Table 23.1.

**Table 23.1** Organisms living within pitcher microcosms. Based on Table S2 from Adlassnig et al., (2011), with modifications and updates. For metabarcoding studies only the top reported organisms are included; bacteria are reported at the family level.

Taxon	Family or species	Host species	Ref.*	
Bacteria	Actinobacteria	Brevibacteriaceae	<i>N. hemsleyana</i>	1
		Conexibacteraceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2
		Corynebacteriaceae	<i>N. hemsleyana</i> , <i>N. rafflesiana</i>	1
		Dermabacteraceae	<i>N. hemsleyana</i>	1
		Dietziaceae	<i>N. hemsleyana</i>	1
		Microbacteriaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i> , <i>N. sp.</i>	2, 3
		Mycobacteriaceae	<i>N. albomarginata</i> , <i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. hemsleyana</i> , <i>N. mirabilis</i> var. <i>echinostoma</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. smilesii</i> , <i>N. stenophylla</i> , <i>N. suratensis</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	1, 2, 4, 5
		Propionibacteriaceae	<i>N. albomarginata</i> , <i>N. ampullaria</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> var. <i>echinostoma</i>	4

(continued)

**Table 23.1** (Continued)

Taxon	Family or species	Host species	Ref.*	
Bacteroidetes	Bacteroidaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2	
	Chitinophagaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. hemsleyana</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. smilesii</i> , <i>N. stenophylla</i> , <i>N. suratensis</i> , <i>N.</i> <i>tentaculata</i> , <i>N. veitchii</i>	1, 2, 5	
	Cryomorpaceae	<i>N. albomarginata</i> , <i>N. ampullaria</i> , <i>N. hemsleyana</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> 1, 4 var. <i>echinostoma</i>	1, 4	
	Cytophagaceae	<i>N. hemsleyana</i>	1	
	Flavobacteriaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i> , <i>N. sp.</i>	2, 3	
	Porphyromonadaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2	
	Sphingobacteriaceae	<i>N. albomarginata</i> , <i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N.</i> <i>hemsleyana</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> var. <i>echinostoma</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. reinwardtiana</i> , <i>N. rafflesiana</i> , <i>N.</i> <i>smilesii</i> , <i>N. stenophylla</i> , <i>N. suratensis</i> , <i>N. tentaculata</i> , <i>N. veitchii</i> , <i>N. sp.</i>	1, 2, 3, 4, 5	
	Weeksellaceae	<i>N. hemsleyana</i>	1	
	Firmicutes	Bacillaceae	<i>N. rafflesiana</i> , <i>N. sp.</i>	1, 3
		Clostridiaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2
Staphylococcaceae		<i>N. hemsleyana</i> , <i>N. rafflesiana</i>	1	
Streptococcaceae		<i>N. hemsleyana</i>	1	
Proteobacteria	Aeromonadaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2	
	Alcaligenaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i> , <i>N. sp.</i>	2, 3	
	Acetobacteraceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. hemsleyana</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> , <i>N. rafflesiana</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. reinwardtiana</i> , <i>N. smilesii</i> , <i>N. stenophylla</i> , <i>N. suratensis</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	1, 2, 5, 6	
	Bradyrhizobiaceae	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. hemsleyana</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. stenophylla</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	1, 5	
	Burkholderiaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. smilesii</i> , <i>N. stenophylla</i> , <i>N. suratensis</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	1, 2, 5	
	Caulobacteraceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2	
	Comamonadaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. smilesii</i> , <i>N. stenophylla</i> , <i>N. suratensis</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	2, 5	
	Enterobacteriaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. hemsleyana</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. smilesii</i> , <i>N. stenophylla</i> , <i>N.</i> <i>suratensis</i> , <i>N. tentaculata</i> , <i>N. veitchii</i> , <i>N. sp.</i>	1, 2, 3, 5, 6	

(continued)

**Table 23.1** (Continued)

Taxon	Family or species	Host species	Ref.*
	Hyphomicrobiaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2
	Methylocystaceae	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. mirabilis</i>	6
	Methylophilaceae	<i>N. albomarginata</i> , <i>N. ampullaria</i> , <i>N. mirabilis</i> var. <i>echinostoma</i> , <i>N. hirsuta</i>	4
	Moraxellaceae	<i>N. albomarginata</i> , <i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> var. <i>echinostoma</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2, 4
	Nannocystaceae	<i>N. rafflesiana</i>	1
	Neisseriaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2
	Oxalobacteraceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2
	Planctomycetaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2
	Pseudomonadaceae	<i>N. albomarginata</i> , <i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. hemsleyana</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> var. <i>echinostoma</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. rafflesiana</i> , <i>N. smilesii</i> , <i>N. suratensis</i> , <i>N. sp.</i>	1, 2, 3, 4
	Rhodocyclaceae	<i>N. hemsleyana</i>	1
	Rhizobiaceae	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. stenophylla</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	1, 5
	Sphingomonadaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. smilesii</i> , <i>N. stenophylla</i> , <i>N. suratensis</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	1, 2, 5, 6
	Xanthobacteraceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. smilesii</i> , <i>N. suratensis</i>	2, 6
	Xanthomonadaceae	<i>N. ampullaria</i> , <i>N. andamana</i> , <i>N. gracilis</i> , <i>N. hemsleyana</i> , <i>N. hirsuta</i> , <i>N. mirabilis</i> , <i>N. mirabilis</i> var. <i>globosa</i> , <i>N. mirabilis</i> var. <i>mirabilis</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. smilesii</i> , <i>N. stenophylla</i> , <i>N. suratensis</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	1, 2, 5, 6
	Undetermined phyla	<i>Bacterium colianindolicum</i> , <i>Bacterium diffusum</i> , <i>Bacterium gastricum</i>	7
Fungi	Ascomycota	<i>Aspergillus glaucus</i> , <i>Penicillium glaucum</i>	7
		<i>Aureobasidium pullans</i> , <i>Candida diffluens</i>	8
		<i>Candida</i> sp.	5
	Undetermined Herpotrichiellaceae	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. stenophylla</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	5
	Undetermined yeasts	<i>N. madagascariensis</i>	9
	Basidiomycota	<i>Cryptococcus albidus</i> , <i>Tilletiopsis</i> sp.	8

(continued)

**Table 23.1** (Continued)

Taxon	Family or species	Host species	Ref.*
	<i>Bullera alba</i>	<i>N. macfarlanei</i> , <i>N. sanguinea</i>	8
	<i>Cryptococcus laurentii</i> , <i>Trichosporon pullulans</i>	<i>N. macfarlanei</i>	8
	<i>Rhodotorula rubra</i>	<i>N. gracilis</i> , <i>N. macfarlanei</i> , <i>N. sanguinea</i>	8
	<i>Sporobolomyces roseus</i>	<i>N. mirabilis</i> , <i>N. macfarlanei</i> , <i>N. sanguinea</i>	8
	Undetermined Agaricomycetes	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. stenophylla</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	5
Chitridiomycota	Undetermined Monoblepharidales	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. stenophylla</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	5
Myxomycota	<i>Merismopedium glaucum</i>	<i>N. melamphora</i>	10
Zygomycota	<i>Mucor mucido</i> , <i>Mucor</i> <i>racemosus</i> , <i>Rhizopus</i> <i>nigricans</i>	<i>N. mirabilis</i>	7
	Undetermined Mucoromycotina	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. stenophylla</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	5
Algae	Bacilliarophyta <i>Achnanthes lanceolata</i> , <i>Achnanthes minutissima</i> , <i>Cocconeis placentula</i> var. <i>lineata</i> , <i>Epithemia sorex</i> , <i>Navicula elliptica</i> , <i>Navicula</i> <i>viridis</i>	<i>N. melamphora</i>	10
	Chlorophyta <i>Euastrum</i> sp.	<i>N. melamphora</i>	10
	<i>Goniomonas</i> sp.	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	11
	<i>Microthamnion</i> sp., <i>Pseudomuriella</i> sp.	<i>N. ampullaria</i> , <i>N. rafflesiana</i>	11
Protozoa	Amoebozoa <i>Acanthamoeba</i> sp.	<i>N. gracilis</i>	11
	Apicomplexa Undetermined Gregarinasina	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	11
	Cercozoa Undetermined Cercomonas	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	11
	Undetermined Heteromita	<i>N. gracilis</i> , <i>N. rafflesiana</i>	11
	Undetermined Phaeodarea	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	11
	Choanozoa <i>Lagenoecca</i> sp.	<i>N. ampullaria</i> , <i>N. rafflesiana</i>	11
	Ciliophora Undetermined CONthreeP	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	11
	Euglenophyta Undetermined Euglenida	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	11
	Euglenozoa Undetermined Kinetoplastea	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	11
	Rhizopoda <i>Amoeba guttula</i> , <i>Amoeba</i> <i>nepenthesi</i> , <i>Arcella vulgaris</i> , <i>Centropyxis aculeata</i> , <i>Cochliopodium bilimbosum</i> , <i>Diffugia constricta</i> , <i>Lesquereusia epistomium</i>	<i>N. melamphora</i>	10
Rotifera	Bdelloidea Undetermined Philodinidae	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. stenophylla</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	5

(continued)

**Table 23.1** (Continued)

Taxon	Family or species	Host species	Ref.*	
Vermiform	Nematoda	<i>Baujardia mirabilis</i>	<i>N. mirabilis</i>	12
		<i>Dorylaimus</i> sp., <i>Subanguina</i> sp.	<i>N. sp.</i>	13
		Undetermined nematodes	<i>N. alata</i>	14
	Oligochaeta	<i>Aeolosoma</i> sp.	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	11
		Undetermined Naididae	<i>N. ampullaria</i>	11
		Undetermined Oligochaeta	<i>N. sp.</i>	15
Crustacea	Copepoda	<i>Epactophanes richardi</i>	<i>N. ampullaria</i> , <i>N. sp.</i>	15, 16
		<i>Parastenocaris incerta</i> , <i>Phyllognathopus viguieri</i>	<i>N. ampullaria</i>	16
		<i>Phyllognathopus viguieri</i> ssp. <i>menzeli</i>	<i>N. sp.</i>	16
		Undetermined Cyclopoida	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. stenophylla</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	5
		Undetermined Harpacticoida	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. stenophylla</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	5, 17
	Decapoda	<i>Geosesarma malayanum</i>	<i>N. ampullaria</i> , <i>N. bicalcarata</i>	18
Undetermined Decapoda Dutch "Garneeltjes"		<i>N. cf. mirabilis</i>	19, 20	
	Ostracoda	<i>Cypridiopsis</i> sp.	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. hirsuta</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. stenophylla</i> , <i>N. tentaculata</i> , <i>N. veitchii</i>	5
Arachnida	Acari	<i>Anoetus nepenthesiana</i>	<i>N. albomarginata</i> , <i>N. gracilis</i> , <i>N. ampullaria</i> , <i>N. gymnamphora</i> , <i>N. mirabilis</i> , <i>N. tobaica</i>	21
		<i>Creutzeria seychellensis</i>	<i>N. pervillei</i>	22
		<i>Creutzeria</i> sp.	<i>N. madagascariensis</i>	9
		<i>Creutzeria tobaica</i>	<i>N. albomarginata</i> , <i>N. madagascariensis</i> , <i>N. mirabilis</i> , <i>N. reinwardtiana</i> , <i>N. tobaica</i>	21, 22
		<i>Hormosianoetus</i> sp.	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	11
		<i>Nepenthacarus warreni</i>	<i>N. mirabilis</i>	23
		<i>Rostrozetes</i> sp.	<i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	11
		<i>Zwickia guentheri</i>	<i>N. albomarginata</i> , <i>N. ampullaria</i> , <i>N. distillatoria</i> , <i>N. gracilis</i> , <i>N. gymnamphora</i> , <i>N. mirabilis</i>	13, 21
		<i>Zwickia nepenthesiana</i>	<i>N. ampullaria</i> , <i>N. gracilis</i>	13
		Undetermined Anoetidae	<i>N. alata</i>	14
Araneae	<i>Misumenops nepenthicola</i>	<i>N. albomarginata</i> , <i>N. gracilis</i> , <i>N. gymnamphora</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. tobaica</i>	13, 21, 22	
	<i>Misumenops thienemanni</i>	<i>N. tobaica</i>	21	
	<i>Peucetia</i> sp., <i>Theridion</i> <i>decaryi</i> , <i>Thyena</i> sp., <i>Synaema</i> <i>obscuripes</i>	<i>N. madagascariensis</i>	9, 24	
	<i>Theridion</i> sp.	<i>N. stenophylla</i>	21	
	<i>Thomisus callidus</i>	<i>N. tobaica</i>	21	

(continued)

**Table 23.1** (Continued)

Taxon	Family or species	Host species	Ref. *
	<i>Thomisus nepenthophilus</i>	<i>N. mirabilis</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. tobaica</i> , <i>N. sp.</i>	13, 21
	Undetermined spiders	<i>N. mirabilis</i>	25
Insects	Collembola	<i>Podura aquatic</i>	10
	Odonata	<i>Lyriothemis salva</i>	26
	Lepidoptera	<i>Eublemma radda</i>	21
		<i>Nepenthophilus tigrinus</i>	21
		<i>Phyllocnistis nepenthae</i>	21
	Hymenoptera	<i>Allocata</i> sp.	21
		<i>Camponotus</i> sp.	27
		<i>Camponotus schmitzi</i>	21, 28
		<i>Dolichoderus bituberculatus</i>	21
		<i>Polyrachis nepenthicola</i>	29
		<i>Tachinaephagus</i> sp.	21
		<i>Trichopria</i> sp.	21
		Undetermined Elachertida	21
		Undetermined Encyrtida	21
	Diptera	<i>Aedes albopictus</i>	17
		<i>Aedes brevitibia</i>	17, 21
		<i>Aedes dybasi</i> , <i>A. maehleria</i>	30
		<i>Aedes gani</i> , <i>A. medialis</i>	21
		<i>Aedes treubi</i>	21
		<i>Armigeres conjugens</i> , <i>A. flavus</i> , <i>A. hybridus</i> , <i>A. malati</i>	21
		<i>Armigeres durami</i> , <i>A. giveni</i> , <i>A. kuchingensis</i>	21
		<i>Armigeres magnus</i>	21
		<i>Corethrella calathicola</i>	17
		<i>Corethrella</i> spp.	31, 32
		<i>Culex acutipalus</i> , <i>C. hewitti</i>	17, 21
		<i>Culex coeruleus</i>	13, 17, 31, 32
		<i>Culex curtipalpis</i>	14, 21
		<i>Culex eminentia</i>	21
		<i>Culex jenseni</i>	21
		<i>Culex lucaris</i>	21
		<i>Culex navalis</i>	14, 17, 21
		<i>Culex shebbearei</i> , <i>C. sumatranus</i>	21

(continued)

**Table 23.1** (Continued)

Taxon	Family or species	Host species	Ref.*
	<i>Culicoides confinis</i>	<i>N. mirabilis</i>	15
	<i>Dasyhelea ampullariae</i>	<i>N. ampullaria</i> , <i>N. gracilis</i>	21
	<i>Dasyhelea biseriata</i>	<i>N. ampullaria</i>	21
	<i>Dasyhelea confinis</i> , <i>D. subgrata</i>	<i>N. mirabilis</i>	21
	<i>Dasyhelea nepenthicola</i>	<i>N. ampullaria</i> , <i>N. albomarginata</i> , <i>N. gracilis</i>	21
	<i>Dasyhelea</i> sp.	<i>N. ampullaria</i> , <i>N. bicalcarata</i> , <i>N. alata</i> , <i>N. rafflesiana</i> , <i>N. mirabilis</i> , <i>N. albomarginata</i>	14, 15, 17, 31, 32
	<i>Endonepenthia cambodiae</i>	<i>N. ampullaria</i>	21
	<i>Endonepenthia campylonympha</i>	<i>N. mirabilis</i>	21
	<i>Endonepenthia gregalis</i>	<i>N. gymnamphora</i>	21
	<i>Endonepenthia schuitemakeri</i>	<i>N. albomarginata</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	13, 21
	<i>Endonepenthia</i> spp.	<i>N. ampullaria</i> , <i>N. sp.</i>	17, 22
	<i>Endonepenthia tobaica</i>	<i>N. tobaica</i>	21
	<i>Forcipomyia</i> sp.	<i>N. ampullaria</i>	17
	<i>Lestodiplosis</i> sp.	<i>N. ampullaria</i> , <i>N. alata</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> , <i>N. rafflesiana</i>	13, 14, 17, 33
	<i>Lestodiplosis syringopais</i>	<i>N. albomarginata</i> , <i>N. gracilis</i> , <i>N. tobaica</i>	21
	<i>Megarhinus metallicus</i>	<i>N. sanguinea</i>	22
	<i>Megaselia</i> cf. <i>bivesicata</i>	<i>N. ampullaria</i>	21
	<i>Megaselia cambodiae</i>	<i>N. sp.</i>	15
	<i>Megaselia campylonympha</i>	<i>N. ampullaria</i> , <i>N. mirabilis</i>	15
	<i>Megaselia corkerae</i>	<i>N. mirabilis</i>	22
	<i>Megaselia decipiens</i>	<i>N. gymnamphora</i>	21
	<i>Megaselia deningsi</i> , <i>M. meningi</i>	<i>N. distillatoria</i>	21, 22
	<i>Megaselia gregalis</i>	<i>N. distillatoria</i> , <i>N. gymnamphora</i>	15
	<i>Megaselia nepenthina</i>	<i>N. albomarginata</i> , <i>N. gracilis</i> , <i>N. mirabilis</i> , <i>N. sp.</i>	15, 21
	<i>Megaselia schuitemakeri</i>	<i>N. albomarginata</i> , <i>N. gracilis</i>	22
	<i>Megaselia</i> spp.	<i>N. ampullaria</i> , <i>N. alata</i>	14, 17
	<i>Megaselia tobaica</i>	<i>N. tobaica</i>	15
	<i>Metricnemus</i> sp.	<i>N. ampullaria</i> , <i>N. tentaculata</i> , <i>N. cf. villosa</i> , <i>N. sp.</i>	15, 21
	<i>Mimomyia jeansottei</i>	<i>N. madagascariensis</i>	21
	<i>Nepenthomyia malayana</i>	<i>N. ampullaria</i>	22

(continued)



Table 23.1 (Continued)

Taxon	Family or species	Host species	Ref.*
	<i>Nepenthosyrphus</i> cf. <i>capitatus</i>	<i>N. albomarginata</i> , <i>N. reinwardtiana</i> , <i>N. tobaica</i>	21
	<i>Nepenthosyrphus malayanus</i> , <i>N. venustus</i>	<i>N. sp.</i>	21
	<i>Nepenthosyrphus oudemansi</i>	<i>N. ampullaria</i> , <i>N. rafflesiana</i>	21
	<i>Nepenthosyrphus</i> spp.	<i>N. ampullaria</i> , <i>N. bicalcarata</i>	17, 31, 32
	<i>Phaonia nepenthicola</i>	<i>N. gymnamphora</i>	21
	<i>Phaonia</i> sp.	<i>N. alata</i>	14
	<i>Pierretia urceola</i>	<i>N. albomarginata</i> , <i>N. gracilis</i>	21, 22
	<i>Polypedilum convexum</i>	<i>N. bicalcarata</i> , <i>N. ampullaria</i>	15
	<i>Polypedilum</i> sp.	<i>N. ampullaria</i> , <i>N. bicalcarata</i> , <i>N. cf. villosa</i> , <i>N. sp.</i>	13, 15, 31, 32
	<i>Sarcophaga</i> sp.	<i>N. sanguinea</i>	21
	<i>Sarcosolomonina pauensis</i>	<i>N. mirabilis</i>	15
	<i>Sarcosolomonina carolinensis</i>	<i>N. sp.</i>	15
	<i>Succingulum fransseni</i>	<i>N. mirabilis</i>	21
	<i>Systemus</i> spp.	<i>N. ampullaria</i> , <i>N. bicalcarata</i>	31, 32
	<i>Toxorhynchites acaudatus</i>	<i>N. ampullaria</i> , <i>N. rafflesiana</i>	21
	<i>Toxorhynchites ater</i>	<i>N. rafflesiana</i>	21
	<i>Toxorhynchites aurifluus</i> , <i>T. coeruleus</i> , <i>T. nepenthicola</i> , <i>T. nepenthes</i> , <i>T. nigripes</i> , <i>T. pendleburyi</i> , <i>T. quasiferox</i> , <i>T. sumatranus</i> ,	<i>N. sp.</i>	21, 22
	<i>Toxorhynchites indicus</i>	<i>N. ampullaria</i>	17
	<i>Toxorhynchites klossi</i>	<i>N. albomarginata</i>	21
	<i>Toxorhynchites metallicus</i>	<i>N. sanguinea</i>	21
	<i>Toxorhynchites</i> sp.	<i>N. ampullaria</i> , <i>N. bicalcarata</i> , <i>N. rafflesiana</i> , <i>N. alata</i>	13, 14, 31, 32
	<i>Toxorhynchites splendens</i>	<i>N. ampullaria</i> , <i>N. rafflesiana</i>	21
	<i>Tripteroides adentata</i> , <i>T. apoensis</i> , <i>T. barraudi</i> , <i>T. belkini</i> , <i>T. bimaculipes</i> , <i>T. christophersi</i> , <i>T. cuttsi</i> , <i>T. delpilari</i> , <i>T. digoelensis</i> , <i>T. dyari</i> , <i>T. elegans</i> , <i>T. flabelliger</i> , <i>T. intermediatus</i> , <i>T. longipalpatus</i> , <i>T. malvari</i> , <i>T. mathesoni</i> , <i>T. mendacis</i> , <i>T. microcala</i> , <i>T. microlepis</i> , <i>T. obscurus</i> , <i>T. pallidus</i> , <i>T. papua</i> , <i>T. pillosus</i> , <i>T. reiseni</i> , <i>T. roxasi</i> , <i>T. simplex</i> , <i>T. simulatus</i> , <i>T. wernerii</i>	<i>N. sp.</i>	21
	<i>Tripteroides aranoides</i>	<i>N. sanguinea</i>	22

(continued)

**Table 23.1** (Continued)

Taxon	Family or species	Host species	Ref.*
	<i>Tripteroides bambusa</i>	<i>N. albomarginata</i> , <i>N. ampullaria</i> , <i>N. gracilis</i>	21
	<i>Tripteroides bisquamatus</i> , <i>T. brevirhynchus</i> , <i>T. caledonicus</i> , <i>T. filipes</i> , <i>T. kingi</i> , <i>T. subobscurus</i>	<i>N. mirabilis</i>	21, 22
	<i>Tripteroides dofleini</i>	<i>N. distillatoria</i>	21
	<i>Tripteroides nepenthicola</i>	<i>N. alata</i>	21
	<i>Tripteroides nepenthis</i>	<i>N. ampullaria</i> , <i>N. bicalcarata</i> , <i>N. rafflesiana</i>	17, 21, 31, 32
	<i>Tripteroides nepenthisimilis</i>	<i>N. ampullaria</i> , <i>N. rafflesiana</i>	21
	<i>Tripteroides</i> spp.	<i>N. ampullaria</i> , <i>N. alata</i>	14, 17
	<i>Tripteroides tenax</i>	<i>N. albomarginata</i> , <i>N. ampullaria</i> , <i>N. gracilis</i> , <i>N. gymnamphora</i> , <i>N. rafflesiana</i> , <i>N. reinwardtiana</i> , <i>N. sanguinea</i> , <i>N. tobaica</i>	17, 21
	<i>Tripteroides vicinus</i>	<i>N. gracilis</i> , <i>N. lowii</i> , <i>N. mirabilis</i> , <i>N. sanguinea</i>	21, 22
	<i>Uranotaenia ascidiicola</i>	<i>N. gymnamphora</i>	21
	<i>Uranotaenia belkini</i> , <i>U. bosseri</i> , <i>U. brunhesi</i> , <i>U. damasci</i>	<i>N. madagascariensis</i>	9, 21
	<i>Uranotaenia gigantea</i>	<i>N. alata</i> , <i>N. tobaica</i>	14, 21
	<i>Uranotaenia moultoni</i>	<i>N. ampullaria</i> , <i>N. bicalcarata</i> , <i>N. alata</i> , <i>N. gracilis</i> , <i>N. rafflesiana</i>	17, 21, 31, 32
	<i>Uranotaenia nepenthes</i>	<i>N. pervillei</i>	22
	<i>Uranotaenia nivipleura</i>	<i>N. distillatoria</i>	22
	<i>Uranotaenia</i> sp.	<i>N. ampullaria</i>	17
	<i>Uranotaenia xanthomelaena</i>	<i>N. gracilis</i>	21
	<i>Wilhelmia nepenthicola</i>	<i>N. alata</i> , <i>N. ampullaria</i> , <i>N. rafflesiana</i>	14, 21
	<i>Xenoplatyura beaveri</i>	<i>N. ampullaria</i>	13
	<i>Xylota</i> sp.	<i>N. ampullaria</i>	21
	Undetermined Anoetidae	<i>N. albomarginata</i> , <i>N. ampullaria</i>	17, 21
	Undetermined Chironomidae	<i>N. ampullaria</i>	17
	Undetermined Chloropidae	<i>N. madagascariensis</i>	9
	Undetermined Dolichopodidae	<i>N. ampullaria</i>	15
	Undetermined Lauxanidae	<i>N. sp.</i>	21
	Undetermined Muscomorphae	<i>N. alata</i>	14
	Undetermined Sarcophagidae	<i>N. maxima</i>	15
	Undetermined Sciarida	<i>N. ampullaria</i> , <i>N. alata</i>	14, 17
Vertebrates Amphibia	<i>Kalophrynus pleurostigma</i> ssp. <i>pleurostigma</i>	<i>N. ampullaria</i>	34, 35

(continued)

**Table 23.1** (Continued)

Taxon	Family or species	Host species	Ref.*
	<i>Heterixalus tricolor</i>	<i>N. madagascariensis</i>	9
	<i>Dendrobates</i> spp.	<i>N.</i> spp.	15
	<i>Microhyla borneensis</i>	<i>N.</i> sp.	34
	<i>Microhyla nepenthicola</i>	<i>N. ampullaria</i>	36
	<i>Phyllautus aurifasciatus</i> (dubious description)	<i>N. sanguinea</i>	34
	Undetermined tadpoles	<i>N. ampullaria</i> , <i>N. bicalcarata</i>	31, 32

\*References: 1. Sickel et al. (2016); 2. Kanokratana et al. (2016); 3. Chan et al. (2016); 4. Takeuchi et al. (2015); 5. Bittleston (2016); 6. Chou et al. (2014); 7. Okahara (1933); 8. Shivas and Brown (1989); 9. Ratsirarson and Silander (1996); 10. Van Oye (1921); 11. Bittleston et al. (2016a); 12. Bert et al. (2003); 13. Choo et al. (1997); 14. Sota et al. (1998); 15. Kitching (2000); 16. Reid (2002); 17. Mogi and Yong (1992); 18. Carrow et al. (1997); 19. Beekmann (2004); 20. Rumphius (1750); 21. Beaver (1983); 22. Juniper et al. (1989); 23. Fashing (2002); 24. Rembold et al. (2013); 25. Hua and Li (2005); 26. Corbet (1983); 27. Clarke and Kitching (1995); 28. Barthlott et al. (2004); 29. Grafe and Kohout (2013); 30. Mogi (2010); 31. Cresswell (1998); 32. Cresswell (2000); 33. Clarke and Kitching (1993); 34. Lim and Ng (1991); 35. Ming (1997); 36. Das and Haas (2010).

### 23.3 Physical properties of *Nepenthes* pitchers

A living, growing pitcher creates the habitat for its inquiline community, and its properties influence the colonization and establishment of the inquilines. Pitchers attract prey with both olfactory and visual cues, and these cues also likely influence initial colonization of inquilines (Moran et al. 1999, Di Giusto et al. 2008, Bazile et al. 2015). Extrafloral nectar and pitcher fluid both produce volatile scents that attract arthropod prey and perhaps adult inquilines looking to oviposit in an appropriate habitat (Di Giusto et al. 2008). Likewise, ultraviolet reflectance around the pitcher rim and pitcher coloration or structure serve as prey attractants and possibly as signals to inquilines (Moran et al. 1999, Kurup et al. 2013). Microbial organisms are unlikely to use olfactory or visual cues for pitcher colonization, but bacteria, fungi, and protozoa may enter pitchers on or in arthropod prey or inquilines, and thus might be indirectly affected by these pitcher properties.

Once organisms have reached pitchers, internal properties of the fluid affect their establishment. Pitcher-fluid pH varies with species, age, prey content, decomposition, and possibly rainfall (Kitching 2000, Adlassnig et al. 2011, Bazile et al. 2015). Oxygen levels in pitcher fluid are higher than in the same fluids transferred to inert tubes, most likely because of photosynthesis by the pitcher itself (Juniper

et al. 1989, Adlassnig et al. 2011). Oxygen content may influence the viability of arthropod inquilines in *Nepenthes*, and should alter the relative abundances of aerobic and anaerobic bacteria. *Nepenthes* pitcher fluid contains free radicals generated from hydrogen peroxide that likely create a harsher environment for inquilines (Chia et al. 2004, Adlassnig et al. 2011). Certain *Nepenthes* species, for example *N. rafflesiana*, *N. fusca*, and *N. maxima*, can produce very viscous fluids (Bonhomme et al. 2011b; Chapter 15), but the effects of viscosity on pitcher inquiline communities are unstudied.

### 23.4 *Nepenthes* inquilines and their functional roles

Some inquilines colonize pitchers opportunistically, whereas many others are specialists that are limited to pitchers for at least one stage of their life cycles (Beaver 1983). Specialist inquilines likely have adapted to pitcher cues and conditions, and take advantage of a protected habitat that attracts external nourishment in the form of insect prey.

#### 23.4.1 Arthropods, vermiform organisms, and rotifers

**Taxonomic composition.** Aquatic insects and mites frequently colonize *Nepenthes* pitchers and often

specialize on these unusual habitats. Diptera are the most obvious inquilines; the most common are mosquitoes (Culicidae), midges (Chironomidae, Ceratopogonidae, Cecidomyiidae, Chaoboridae) and flies in four other families (Phoridae, Calliphoridae, Syrphidae, and Sarcophagidae). Common genera of *Nepenthes* mosquitoes include *Armigeres*, *Culex*, *Toxorhynchites*, *Tripteroides*, and *Uranotaenia* (Beaver 1983, Clarke and Kitching 1993). Occasional *Aedes* mosquitoes occur as *Nepenthes* inquilines, but these have different traits from non-pitcher *Aedes* spp. and do not feed on human blood (Mogi 2010). In particular, pitchers are not suitable habitats for *Aedes albopictus* (Chou et al. 2015) and are not attractive to gravid females of *A. albopictus* or *A. aegypti* (Chou et al. 2016), so *Nepenthes* are unlikely to serve as breeding grounds for vectors of human disease.

Mites (Histiostomatidae and Acaridae) also are very common. Crustacea, vermiforms (worms and worm-like animals), and rotifers are sporadically collected. From a metabarcoding study using Illumina sequencing, the most abundant crustaceans were harpacticoid and cyclopoid copepods and podocopic ostracods (Bittleston 2016). The most abundant vermiform operational taxonomic unit (OTU) matched to Aeolosomatidae, a family of micro-annelids that live in soil and decaying matter in stagnant water (Bittleston 2016). Other abundant annelid OTUs were assigned to the Naididae (detritus worms). A sequence classified as a Platyhelminthes (flatworm) was very abundant and might represent an inquiline parasite.

Nematode OTUs, present in about 25% of sampled pitchers, were taxonomically assigned principally to the Panagrolaimidae and Rhabditidae. Bert et al. (2003) isolated a new species of nematode from this family and described a new genus based on individuals found in *Nepenthes mirabilis* in Thailand. Choo et al. (1997) also noted two species of nematodes living in the pitchers of Singaporean *Nepenthes*, and undetermined species of nematodes were found in 11% of the *N. alata* pitchers examined in West Sumatra (Sota et al. 1998). Thienemann (1932) noted rotifers and Beaver (1983) classified them as occasional nepenthexenes, but they actually may play a significant role in the pitcher ecosystem. For example, in *Sarracenia purpurea* pitchers, the

bdelloid rotifer *Habrotrocha rosa* is a common inquiline and contributes significant amounts of nitrogen and phosphorus to the plant's nutrient budget (Błędzki and Ellison 1998). In the metabarcoding dataset, abundant OTUs close to unnamed bdelloid rotifers in the Philodinidae were present in ≈30% of the sampled pitchers (Bittleston 2016).

**Ecological functions.** The macroscopic organisms living in *Nepenthes* pitchers generally are classified as saprophages or predators. The saprophages include filter feeders that feed on living or dead matter suspended in the water column, and detritivores that feed on settled detritus (Kitching 2000). Direct observations of feeding are rare, and the functional roles of inquilines often are extrapolated from close relatives. Aedine and anopheline mosquitoes, rotifers, copepods, and histiostigmatid mites are filter feeders that likely consume protozoa, bacteria, and suspended particles (Kitching 2000). Nematodes, ostracods, culicine mosquitoes, chironomid and ceratopogonid midges, phorid flies, and astigmatid mites are thought to feed primarily on fine detritus and any attached microbes. Calliphorid and sarcophagid flies feed on larger detritus and exploit recent prey, but also may act as predators (Sota et al. 1998).

*Toxorhynchites* mosquitoes and chaoborid midges are voracious predators of other insect larvae. Cannibalism has been observed in inquiline *Toxorhynchites* spp., and generally only one individual is present per pitcher (Beaver 1983). Other genera of mosquitoes, including *Culex* and *Topomyia*, may be facultative predators. Cecidomyiid midges are specialist predators of phorid fly larvae and other small dipterans (Clarke and Kitching 1993).

### 23.4.2 Fungi, protozoa, algae, and bacteria

**Taxonomic composition.** Fungi, protozoa, algae, and bacteria are common and abundant inquilines of all *Nepenthes* species examined to date. Microbial inquilines initially were studied in the early-to-mid 1900s (Hepburn 1918, Van Oye 1921, Okahara 1933) when classification of microbes was difficult. In recent years, “next-generation” sequencing and metabarcoding has improved greatly the ability to characterize and classify microbes (Baker et al. 2016, Bittleston et al. 2016a). Metabarcoding is extremely

useful for uncovering microscopic or cryptic organisms but species identification is difficult because it relies on existing databases. DNA sequences of most pitcher organisms are not present in the databases and many represent new species, so exact identification is impossible and there is still a strong need for good morphological classification based on descriptive zoology and microbiology.

*Nepenthes* species produce naphthoquinones with anti-fungal activity (Cannon et al. 1980, Shin et al. 2007a, Eilenberg et al. 2010), but fungi—particularly yeasts—still thrive within pitchers. Pitcher-fluid cultures from four *Nepenthes* species in West Malaysia yielded several different yeast species (Shivas and Brown 1989), and metabarcoding of eight *Nepenthes* species from Singapore and Borneo found *Candida* yeasts (Saccharomycetales) to be the most abundant fungi (Bittleston 2016, Bittleston et al. 2016a). Metabarcoding studies also found numerous basal fungi, including chitrids (Monoblepharidales) and several Mucoromycotina. Other relatively abundant fungal OTUs included an ascomycete with strong matches to the Herpoticchiellae (Pezizomycotina) and a basidiomycete (Agaricomycetes) (Bittleston 2016). Fungi live within pitcher tissue as endophytes as well as inside the fluid. A recent study isolated 26 endophytic fungi from *N. ampullaria* and *N. mirabilis* pitchers and leaves, mainly from the *Colletotrichum* species complex (Lee et al. 2014).

Protists are common in *Nepenthes* pitchers. Metabarcoding has revealed that most abundant protozoan inquilines are ciliates (Ciliophora), flagellates (Euglenophyta and Cercozoa), gregarines (Apicomplexa), and amoebae (multiple lineages) (Bittleston 2016, Bittleston et al. 2016a). In addition to protozoa, *Nepenthes* pitcher fluids can have high abundances of green algae (Chlorophyceae) (Bittleston 2016, Bittleston et al. 2016a). Green algae photosynthesize, and thus act as primary producers, adding organic carbon to the pitcher fluid ecosystems to be consumed by other inquilines. Algae may be present in pitcher fluid even when it is not noticeably green, and so are not always obvious to the naked eye.

In terms of numbers, bacteria are probably the most abundant and diverse organisms living in pitcher fluid. In a metabarcoding study of the 16S ribosomal RNA gene, the bacterial orders with the

highest relative abundances were Rhodospirillales, Rhizobiales, Actinomycetales, Xanthomonadales, Neisseriales, and the family Chitinophagaceae in an uncertain order (Bittleston 2016, Bittleston et al. 2016a). Sickel et al. (2016) also found Rhodospirillales, Actinomycetales, and Rhizobiales as the top three represented orders in their study of *N. rafflesiana* and *N. hemsleyana* pitcher fluid. Two other recent studies from Malaysia and Thailand also report similar taxa, with highly abundant genera from the same phylogenetic groups (Chou et al. 2014, Kanokratana et al. 2016). Takeuchi et al.'s (2015) study does not show similar most-abundant taxa, but they took many of their samples from cultivated plants in Zurich, and did not report data from Bornean field samples separately from European *in vitro* ones.

Pitcher fluid has long been considered to be sterile before the lids open (Hepburn 1918, Okahara 1933). Sterility of unopened pitchers has been well-demonstrated for *Sarracenia purpurea*, the convergently evolved North American purple pitcher plant (Peterson et al. 2008; Chapter 9), but Chou et al. (2014) and Takeuchi et al. (2015) sequenced bacteria from unopened *Nepenthes* pitcher fluid, and Kanokratana et al. (2016) detected bacterial DNA in three of 14 closed *Nepenthes* pitchers. Sota et al. (1998) saw bacteria in their microscopy counts from fluid of unopened pitchers, but noted they could not rule out the possibility of contamination. Buch et al. (2013) did not find bacteria in unopened *Nepenthes* pitchers, and deemed the secreted plant fluid unsuitable for microbial growth. It remains to be definitively determined if unopened *Nepenthes* pitchers are in fact sterile, or if they contain bacteria of internal plant origin.

**Ecological functions.** Most bacteria and fungi in pitchers act as decomposers, breaking down the proteins, fats, and carbohydrates of insect prey and occasional plant material that falls into pitchers. They can secrete extracellular digestive enzymes that likely act in concert with plant-produced enzymes. Bacterial species isolated from *Nepenthes* pitchers in Malaysia were able to degrade protein, starch, xylan, chitin, and cellulose (Chan et al. 2016).

The microbial inquilines cannot be classified into only one functional group, because different trophic levels are represented. Some protozoa are

predatory—consuming bacteria, fungi, or other protozoa. Others are parasitic, and feed on arthropods or other inquilines. Algae, photosynthetic protozoa, and some bacterial taxa (e.g., photosynthetic Cyanobacteria and nitrogen-fixing Rhizobiales) act as primary producers within pitcher ecosystems. The extent to which they increase levels of organic carbon or nitrogen within the pitchers is currently unknown. Ideally, future *Nepenthes* food webs will incorporate different microbial functional groups, and energy budgets will account for primary production by pitcher inhabitants.

### 23.4.3 Other inquilines

Certain arthropods spend only part of their life history within pitcher fluid, and are clearly parasitic, such as crab spiders and noctuid moths in the genus *Eublemma*. Crab spiders, including *Misumenops nepenthicola* (= *Henriksenia labuanica*) and *Thomisus nepenthophilus* in Southeast Asia and *Synaema obscuripes* in Madagascar, live inside the lip of pitchers and feed on trapped prey or inquilines (Chua and Lim 2012, Rembold et al. 2013). If threatened, they can dive into pitcher pools, hide in the detritus at the bottom, and later climb back out. *Eublemma* moth larvae are specialist herbivores of *Nepenthes* species, and will feed on pitcher tissue—sometimes while partially submerged beneath the fluid. Their feeding can damage pitchers, destroying their water-holding capacity (Dover et al. 1928, Clarke and Kitching 1993).

Vertebrates that live within *Nepenthes* pools are rare, but frogs occasionally colonize pitchers and some frogs even have long-term associations with *Nepenthes* species. For example, one of the world's tiniest frogs, *Microhyla nepenthicola*, breeds only in *N. ampullaria* pitchers in Sarawak, Borneo (Das and Haas 2010). A review by Malkmus and Dehling (2008) includes numerous other instances of frogs colonizing *Nepenthes* pitchers, particularly senescent *N. ampullaria* and, to a lesser extent, *N. bicalcarata*.

### 23.4.4 Inquiline effects on hosts

Inquilines likely both increase and decrease plant-accessible nutrient levels within pitchers, and their

overall effects are unclear. Scharmman et al. (2013) hypothesized that phorid dipterans are kleptoparasites of the *Nepenthes bicalcarata*–*Camponotus schmitzi* mutualism (Chapters 15, 26) and remove nitrogen from the system. When the ants are present, pitchers have higher levels of prey-derived nitrogen and fewer phorid fly adults emerge from the pitchers. This may be because the ants feed on the relatively large phorid flies, decreasing export of nitrogen from pitchers (Scharmman et al. 2013). Conversely, in an *in situ* experiment using fluid and insects from *Nepenthes gracilis*, both culicid and phorid inquilines increased levels of ammonium and soluble protein (Lam et al. 2017). Microbial organisms, in the absence of insects, also increased levels of ammonium. The increases in plant-accessible nitrogen should be beneficial for the plant host. In general, inquilines may both increase and decrease nutrient availability. Insect inquilines increase nutrient availability by ripping apart prey carcasses and making compounds more accessible to extracellular enzymes present in pitcher fluid, and by processing complex proteins and carbohydrates into simpler compounds through feeding and excreting. They decrease nutrient availability by incorporating nutrients into their bodies and removing them from the system when they emerge as adults and leave the pitcher pool. The same dual functions occur for micro-inquilines: bacteria, protozoa, and fungi all consume some resources and excrete others. Their contribution to, and removal from, the total nutrient pool is complex and likely shifts depending on circumstances and environmental conditions. Future experiments should identify nutrient tradeoffs and quantify the extents to which different species (or functional groups) should be considered mutualists, parasites, or commensals.

## 23.5 Geographic patterns

### 23.5.1 Patterns within and among pitchers

Dipteran inquilines have some degree of specificity in the *Nepenthes* species they colonize. Clark and Kitching (1993) examined six different Bornean species of *Nepenthes* that co-occur within 1 km of one another. The inquiline genera found were very



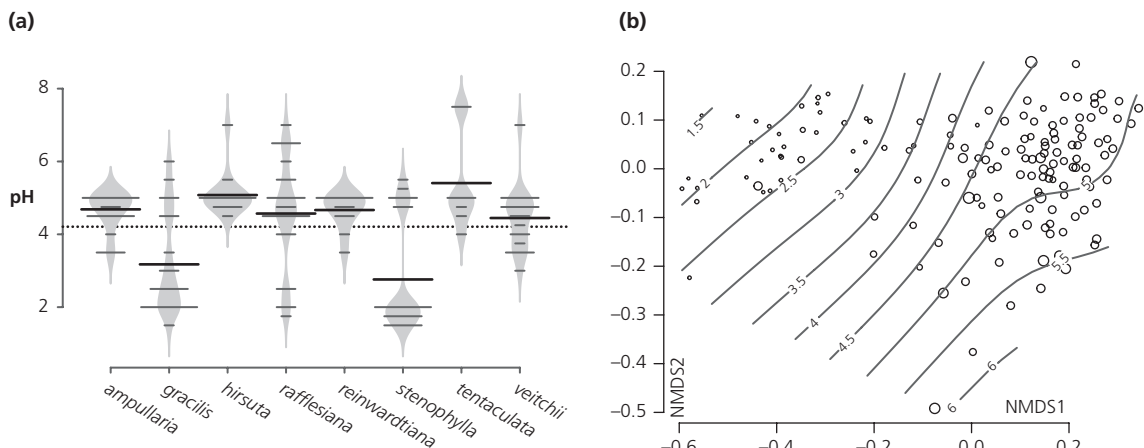
similar to those in *Nepenthes* pitchers in Penang, although few species were shared (Clarke and Kitching 1993). Dipteran inquilines showed preferences for certain species. For example, among the predators, *Lestodiplosis* sp. (Cecidomyiidae) was found only in *N. mirabilis* and *N. gracilis*, whereas *Corethrella* sp. (Chaoboridae) was found only in *N. bicalcarata* and *N. ampullaria* (Clarke and Kitching 1993). Similarly, Bittleston et al. (2016a) found a *Corethrella* sp. only in *N. ampullaria*, and not *N. gracilis* or *N. rafflesiana*. Certain mites also are species-specific: *Naiadacarus nepenthicola* is restricted to the pitchers of *N. bicalcarata*, even when other species grow in close proximity (Fashing and Chua 2002).

Gregarine protozoa are obligate parasites of arthropods. Because they are parasites, one might expect gregarines to echo arthropod community diversity patterns. However, gregarine community diversity patterns depend more on pitcher location, whereas arthropod communities are better explained by identity of the host species (Baker et al. 2016).

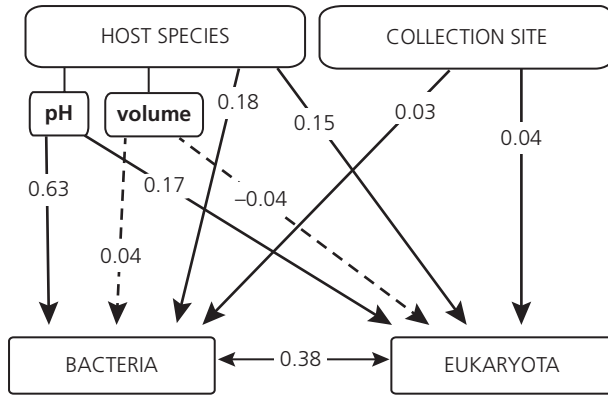
Bacteria have distinct patterns of association with different pitcher plants even when they are in the same habitat, suggesting that certain *Nepenthes* species provide different environmental conditions that create different bacterial niches. Compositional

diversity appears to be driven by different acidity levels and other unmeasured aspects of the *Nepenthes* species, and is less affected by geographic location. *Nepenthes* species differ in their average fluid pH levels, and acidity strongly influences bacterial communities in other habitats (e.g., Fierer and Jackson 2006). In a metabarcoding study across eight *Nepenthes* species from Singapore and Borneo, average fluid pH levels ranged from  $\approx 3$  to 5.5 (Figure 23.1). *N. stenophylla*, *N. gracilis*, and *N. rafflesiana* all had some pitchers with very low pH values (Figure 23.1a). Among measured variables, pH was the best predictor of community composition with a Mantel test (Bittleston 2016; Figures 23.1b, 23.2). A study of the bacterial communities from six species of *Nepenthes* pitchers grown together in an open-air nursery in Thailand also found a correlation between community composition and pitcher pH (Kanokratana et al. 2016).

Kanokratana et al. (2016) found bacterial community composition in *N. ampullaria* differed from that of other species growing together in the same habitat. Across eight *Nepenthes* species from Singapore and Borneo, pitcher-plant species identity was a better predictor than location of community composition (metabarcoding data in Bittleston 2016; Figure 23.2). The volume of pitcher fluid was not



**Figure 23.1** Acidity of *Nepenthes* fluid is different among species and correlates with bacterial community diversity. **(a)** Bean plots of pH in different *Nepenthes* species. The number of samples at each value is represented by the width of the thin lines, and thicker black lines represent mean values for each species. **(b)** Non-metric multidimensional scaling (NMDS) plot of *Nepenthes* bacterial communities using the phylogenetic unweighted UniFrac metric. The size of each circle corresponds to the pH value of each sample, and pH levels are mapped onto the plot as contour lines.



**Figure 23.2** Correlations of measured factors with *Nepenthes* bacterial and eukaryotic communities. Solid lines indicate significant correlations ( $P < 0.05$ ); dashed lines are not significant. The numbers are either Mantel  $r$  values (for continuous variables: pH, volume, and bacteria–eukaryote relationship), or  $r^2$  values from permutational MANOVA tests (for discrete variables: host species and collection site).

significantly correlated with community composition, perhaps because volume changes frequently following rain and evaporation.

Patterns of co-occurrence (or co-presence) and mutual exclusion among inquilines across multiple pitcher samples can indicate species cooperation, competition, or preference for similar conditions (Faust and Raes 2012). It remains unclear whether inquiline community composition simply reflects inherent differences among host species, or if inquilines also contribute to these differences and are increasing niche separation among co-occurring *Nepenthes* species.

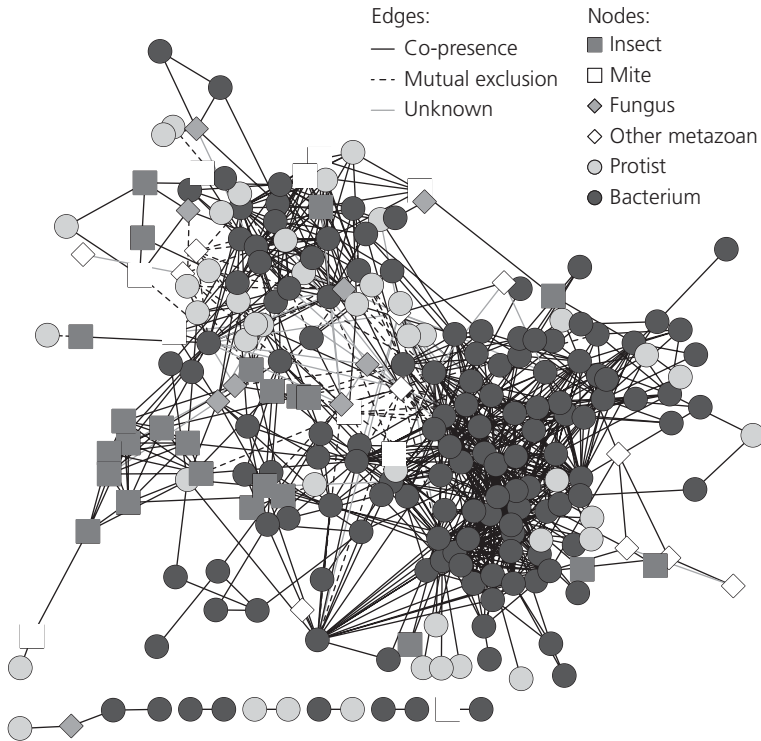
As a first step toward examining these alternative possibilities, I constructed a co-occurrence network of the most abundant inquiline species reported in Bittleston (2016). From OTU tables that were subsampled (rarefied) to the same numbers of sequences per sample (6778 for bacteria and 4852 for eukaryotes), I selected only OTUs with more than either 1000 (bacterial) or 600 (eukaryotic) sequences that were present in at least six pitchers across 175 samples, and combined the bacterial and eukaryotic tables. I used the Co-Net program (Faust et al. 2012) to measure four indices (Pearson and Spearman correlation coefficients, Bray–Curtis dissimilarity, and mutual information) and to retain the top and bottom 1000 edges. I then ran 100 permutations, bootstrapped them, and retained only significant edges (connections between two OTUs with  $\alpha \leq 0.05$  after a Benjamini–Hochberg correction for multiple tests). The resulting network was visualized in Cytoscape, and shows nodes (OTUs) connected by

edges representing either co-presence or mutual exclusion (Figure 23.3).

This co-occurrence network of *Nepenthes* inquilines reveals patterns related to pitcher fluid pH and inquiline identity. Modules, or tightly linked sets of organisms, are present (Figure 23.3). In general, bacteria cluster together most tightly and are surrounded closely by protists. Fungi, arthropods, and other metazoans are on the periphery of the network (Figure 23.3). There are two main clusters of bacteria separated by edges of mutual exclusion: the larger one contains species that are generally more abundant in pitchers with higher pH, and the smaller one contains species generally more abundant in pitchers with low pH. This result reflects other findings and provides further evidence of a strong influence of acidity on bacterial community composition. Insects also form a tight module (Figure 23.3). Insect inquilines may prefer similar conditions in pitchers, thus co-occurring because of shared niche space. That modules contain closely related organisms implies that competition may not be the primary force structuring inquiline communities.

Phylogenetic community compositions of bacteria and eukaryotes were correlated with each other (Figure 23.2). Other than the correlation of bacteria with pH, phylogeny was the strongest associational signal among the measured variables (Bittleston 2016; Figure 23.2). This result suggests that certain bacterial and eukaryotic lineages co-occur either because of real associations among them or because of preferences for similar environmental conditions.





**Figure 23.3** Co-occurrence network of abundant *Nepenthes* inquilines. Nodes represent operational taxonomic units (OTUs) connected either by solid black lines (edges) representing co-occurrence of those organisms, dashed lines representing mutual exclusion, or gray lines where there was not enough information about the relationship.

One possible mechanism for the correlation is introduction of bacteria to pitchers via eukaryotic hosts. However, this hypothesis was not supported: when eukaryotes were subset to arthropods, the correlation was weaker. The co-occurrence network also did not support a strong co-presence among bacteria and arthropods, as they were not attached by many edges (Figure 23.3); instead, I hypothesize that associations among bacteria and protists drive the pattern.

### 23.5.2 Comparisons with surrounding habitats

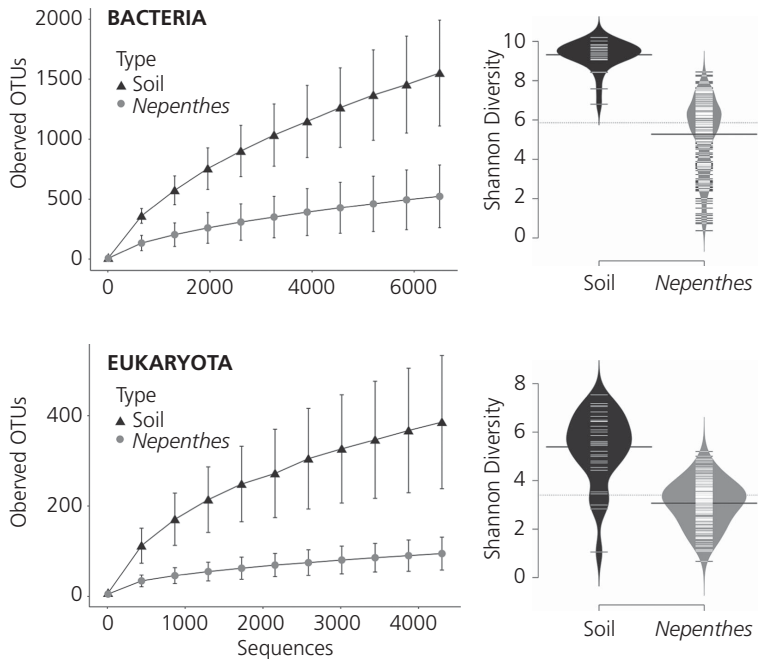
Pitchers are colonized from the surrounding environment, but the inquilines often are different from, or a subset of, species living in surrounding habitats (Bittleston 2016). These data suggest that environmental filtering plays a role in selecting the organisms that colonize and thrive in pitchers.

Inquiline community structure also differs from that of communities living in the surrounding leaf-litter and soil organic layer. Fewer eukaryotes and

bacterial OTUs are found in pitcher plants than in the soil directly around the plants (Figure 23.4). Shannon diversity ( $H'$ ) also is significantly lower ( $P < 0.001$ ) in pitchers than in soil communities, indicating that pitcher communities are less even and less predictable in composition (Figure 23.4). Because pitchers are temporary, ephemeral habitats, it is reasonable that they would contain fewer species and have more variable species composition than surrounding, more stable habitats. Chance also likely plays a role in colonization. Finally, prey capture occurs in pulses, and stochasticity in the availability of nutrients likely contributes further to the variability of inquiline community structure among pitchers.

### 23.5.3 Inquilines of *Nepenthes* and *Sarracenia*

The pitcher habit has evolved at least three times in different lineages of plants: pitchers of Nepenthaceae in Southeast Asia, Sarraceniaceae in the Americas, and Cephalotaceae in Australia all have pitchers that form from a single modified leaf



**Figure 23.4** Species richness and Shannon diversity ( $H$ ) of *Nepenthes* and soil organic layer bacterial (top) and eukaryotic (bottom) communities. *Nepenthes* communities are less species-rich and have lower  $H$  than surrounding soil communities ( $P < 0.001$ ). Error bars in the rarefaction curves represent standard deviations within each sequence-abundance category. The black lines in the bean plots are mean  $H$  values within each group, and the dotted lines are the mean  $H$  of the pooled data.

(Chapters 3, 9, 10). Certain bromeliads from South America are sometimes classified as pitcher plants, although the physical structure of the pitchers is different (Chapter 10). The convergent evolution of pitcher form and function provides an opportunity to examine how similarities in habitat can affect the evolution and maintenance of associated communities (Bittleston et al. 2016b).

I investigated the different families of insects and arachnids living in pitcher habitats, to test if the ones found in *Nepenthes* and *Sarracenia* systems (cf. Chapter 24) were more similar than expected by chance. Using data from Kitching (2000) and Adlassnig et al. (2011), I counted only insect and arachnid families that appeared to be regularly associated with *Nepenthes* or *Sarracenia* species (found more than once in the habitat, avoiding incidental organisms). As a control comparison, I did the same counts for tree-hole inquilines from North America and Southeast Asia. The numbers of inquiline families found in *Sarracenia* and *Nepenthes* were 10 and 21, respectively, with 9 shared between them, whereas for tree holes of North America and Southeast Asia the numbers were 13 and 21, respectively, with 8 shared (respectively,  $P = 0.003$ , and  $P = 0.20$

based on permutation tests with 10,000 randomizations and keeping the number of shared families constant and using all families found in association with phytotelmata as the source population for both pitcher-plant and tree-hole samples). That is, certain families of insects, mites, and spiders appeared more likely to associate with pitcher plants, even on opposite sides of the planet.

Takeuchi et al. (2015) compared *Nepenthes* bacterial communities at the class level to those from other habitats with published data, including farm soil, coastal seawater, the Amazon River, termite gut, human gut, *Arabidopsis thaliana* phyllosphere, and *Sarracenia alata*. They found that *S. alata* bacterial community composition was most similar (Jaccard similarity) to the *Nepenthes* samples. A more extensive study comparing over 140 *Sarracenia* and 180 *Nepenthes* samples substantiated the result: community compositions (using the phylogenetic unweighted UniFrac metric) were more similar to each other than they were to the surrounding bog and soil communities from their respective habitats (Bittleston 2016). Furthermore, both *Nepenthes* and *Sarracenia* pitcher communities had similar measures of observed OTU richness

and  $H'$  for bacteria and eukaryotes (Bittleston 2016). For both macro- and micro-inquilines, the data suggest that convergent evolution of a pitcher appears to lead to convergent interactions among the plants and their associated organisms (Bittleston et al. 2016b).

### 23.6 Future research

The pitchers of carnivorous pitcher plants are living habitats that host complex, diverse communities. Colonizing organisms contend with acidity, viscous fluid, digestive enzymes, and free radicals, but surviving inquilines have a nearly guaranteed source of host-trapped nutrients (prey), higher than normal oxygen levels, and a protective chamber. In general, inquilines are a highly variable subset of the available pool of organisms. Bacterial community structure is strongly correlated with fluid pH, and bacteria co-occur with many protists. Some insect and mite inquilines specialize on certain *Nepenthes* species, potentially because of differences in the physical structure of the pitchers. Because we see some convergence in organisms colonizing *Nepenthes* and *Sarracenia* pitchers, it is likely that pitcher form and function selects for related inquilines in particular functional groups.

Many aspects of *Nepenthes* inquiline communities are still unknown and are prime targets for future research. These include: viral dynamics; succession or

unpredictable community assembly; which (if any) special adaptations are necessary for colonizing pitcher habitats; why some pitcher species acidify their fluid and whether low pH selects for mutualistic bacteria; the extent to which associations are parasitic or mutualistic; and if there are short-term host-inquiline feedback loops or evolutionary co-diversification of inquilines and *Nepenthes*.

Pitchers are ideal model systems for community ecology (Ellison et al. 2003, Srivastava et al. 2004). Because pitcher pools are isolated islands contained within a plant, defining the boundaries of the community is relatively straightforward. The abundance of pitchers and their diminutive size make them convenient ecosystems where one could measure replicate communities across all trophic levels. Future studies could use pitcher systems to measure the predictability of community assembly (e.g., Ellison et al. 2003), and to understand more fully processes that structure communities. New developments in network analyses and other methods for studying complex systems could be used to uncover patterns of species interactions within pitchers, and to examine tipping points in model ecosystems (Sirota et al. 2013). Perhaps studies of pitcher-plant microecosystems will provide a better understanding of general ecosystem dynamics and contribute to conservation efforts in the face of global change (Ellison and Gotelli in press).