

# WANTED



POCA Wong Siu Ching Secondary School

**BUTCHER CATCHER  
PITCHER PLANT**

**£ 80,000,000-**

"Searching For Nature Stories 2013"

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## Abstract

Pitcher plant, one of several insectivorous plants with leaves adapted for trapping insects. In our investigation, a series of observations and experiments were carried out so as to discover how does pitcher plant capture and digest insects. Pitcher plants need to trap insects since they have to obtain extra nutrients, for instance, proteins, nitrogen and other minerals that it could not just seep from the soil where they live. An experiment of testing the presence of protease in the pitcher digestive fluid was taken to prove the fact.

In order to investigate the adaptive features of the pitcher plant, an experiment of dissecting the pitcher cup was conducted. The slippery inner surfaces of the pitchers, which can be waxy or slippery, have so far been considered as the key trapping devices.

The above two experiments are necessary to investigate the mechanism of trapping insects as prey.

## Introduction

A few months ago, we read an article about pitcher plants, a kind of Carnivorous plants when we were searching our investigation target in the project. Later on, we noticed that a pot of pitcher plant was grown in our school garden. So we had an opportunity to take a close look at this extraordinary plant.

### Brief description

This plant is a good example of carnivorous plant for educational and research purposes. In mainland of China the species is only sparsely distributed in marshes of Guangdong and Hainan Province and is endangered by destruction of habitats and over-collection for cultivation as ornamentals. In Hong Kong, some localities of its occurrence are in Country Parks under protection.

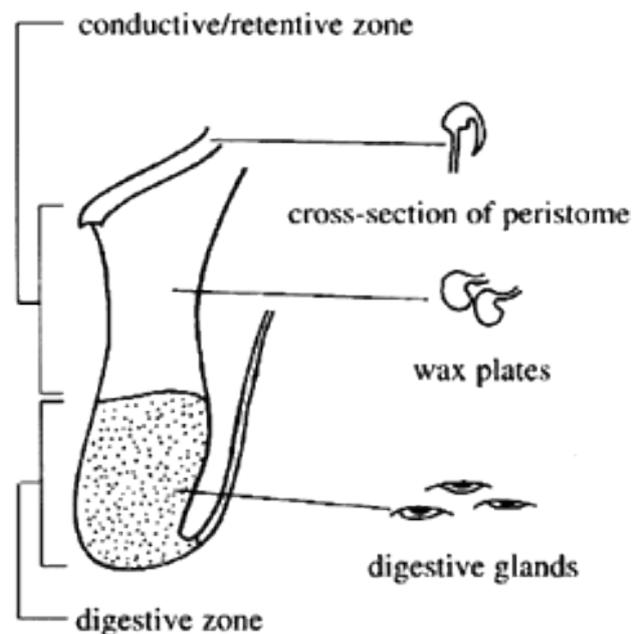
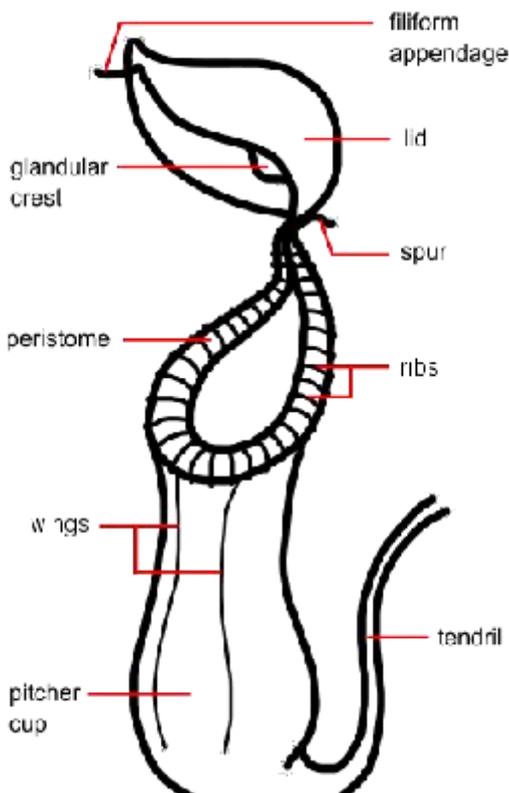
### Profile of Pitcher Plant

	<b>Specific name</b>	Nepenthes mirabilis (Lour.) Druce
	<b>Kingdom</b>	Plantae (Plants)
	<b>Subkingdom</b>	Embryophyta
	<b>Division</b>	Tracheophyta (Vascular Plants)
	<b>Subdivision</b>	Spermatophyta (Seed Plants)
	<b>Class</b>	Angiosperms (Flowering Plants)
	<b>Subclass</b>	Monocotyledons (Monocots)
	<b>Order</b>	Caryophyllales
	<b>Families</b>	Nepenthaceae and Sarraceniaceae
	<b>Genus</b>	Nepenthes
	<b>Diversity</b>	More than 138 species
<b>Distribution</b>	Tai Lam Chung, So Kwun Wat, Castle Peak, Lantau. Hainan, Guangdong; Indochina peninsula, Malaysia	
<b>Habitat and Ecology</b>	In grasses and crevices of marshes	

## Features of Pitcher Plant

	<b>Type</b>	Perennial herbs
	<b>Maximum length</b>	Stem prostrate or climbing, up to 2 m long
	<b>Leaves</b>	<ul style="list-style-type: none"> <li>→ Leaves basal and alternate on stem</li> <li>→ Lanceolate to oblong-lanceolate</li> <li>→ 10-25 cm long and 4-8 cm wide</li> </ul>
	<b>Pitcher cup</b>	Midrib prolonged into a tendril which is greatly expanded and hollowed at end forming the pitcher cup
	<b>Shape of the pitcher</b>	<ul style="list-style-type: none"> <li>→ The pitcher is cylindrical</li> <li>→ 8-16 cm long, 2-5 cm in diam.</li> <li>→ With a orbicular lid at apex</li> </ul>
	<b>Different parts of the plant</b>	→ In a terminal raceme
		→ Pedicels 5-15 mm long
		→ Tepals 4 (-3), elliptic, 4-7 mm long
	<b>Reproduction</b>	→ Capsules ellipsoidal, 15-30 cm
		→ Plants dioecious, flower unisexual
→ In male flower, stamens 4-25 connate into a column		
	→ In female flower, ovary 1, superior, 4-3-celled	

## Structure of Pitcher Plant



## Schedule of our investigation

Year	Date	Venue	Details
2012	17/11 (SAT)	Chinese University of Hong Kong	Opening and training about Searching for Nature Stories
	26/11 (MON)	School Biology Laboratory	Discussion on the topic that we are interested in and is possible to investigate
	17/12 (MON)	School Garden	Observe the plants grow there, notice that pitcher plant is observable
	19/12 (WED)	School Biology Laboratory	Decide pitcher plant as our investigation topic and started to search information
2013	3/1 (THUR)	School Biology Laboratory	Gather information searched and wrote the investigation plan
	21/4 (SUN)	Tai Tong Nature Trail	1 <sup>st</sup> Field investigation: Find pitcher plants and observe its living environment
	27/4 (SAT)	Lions Nature Education Centre	2 <sup>nd</sup> Field investigation: Find pitcher plants and observe its living environment
	29/4 (MON)	School Biology Laboratory	1 <sup>st</sup> Experiment:
	2/5-4/5 (THUR-SAT)	School Biology Laboratory	2 <sup>nd</sup> Experiment: Test the pH value of the pitcher fluid and test the presence of protease in the pitcher plant
	6/5-15/5 (MON-WED)	School Biology Laboratory	Writing the report
	16/5 (THUR)	School Biology Laboratory	Hand in the report

## Field Trip 1

Some of the data in this table are included The Hong Kong Observatory.

<b>Date and Time</b>		22 <sup>nd</sup> April 2013 (Sun)
<b>Venue</b>		Tai Tong Nature Trail
<b>Weather</b>		Cloudy
<b>Duration of sunshine (hrs)</b>		0 hr
<b>Temperature</b>	The highest (°C)	23.9°C
	The lowest (°C)	20.4°C

Route (in red colour):



Photos of the field trip:



### **Observation:**

In this field trip, we walked for around 3 hours (from 9 am to 12 am). We saw a lot of places that were dark and humid which are suitable for pitcher plants to grow. However, due to our carelessness, we could not find any pitcher plants in this field trip.

## Field Trip 2

Some of the data in this table are included The Hong Kong Observatory.

<b>Date and Time</b>		27 <sup>th</sup> April 2013 (Sat)
<b>Venue</b>		AFCD Lions Nature Education Centre, Sai Kung
<b>Weather</b>		Sunny
<b>Duration of sunshine (hrs)</b>		0.6 hr
<b>Temperature</b>	The highest (°C)	24.2°C
	The lowest (°C)	20.5°C

Route (map → with red circle):



Photos of the field trip:

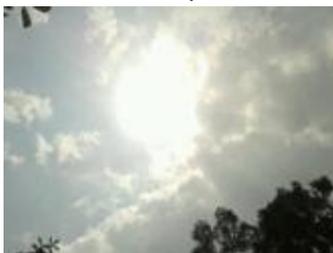


Group photo:



### Observation:

In this field trip, we asked an officer there and she told us where the pitcher plant was. When we went there, we found some of the pitch plants wilted and some of them were just growing. The temperature and evaporation rate there was so high, with the sun shining to the plant directly (**Fig.1**). And the relative humidity is quite low, which is not suitable for pitcher plants. They need to grow in swamps and marshes (*Flora of Hong Kong Volume 1, XIA Nian-he*).



(Fig.1)

## Experiment 1: Pitcher Plant's "eating" process

<b>Question</b>	How does pitcher plant trap insects?
<b>Aim of experiment</b>	(a) To find out how pitcher plants capture insects. (b) The experiment focuses on the adaptive features of their "wall" (wax plate).
<b>Experimental design</b>	We want to find out how the pitcher plants trap insects by their adaptive features. We catch some ants to place them near the edge of the pitcher plants and see how the insects fall into the plants.
<b>Apparatus</b>	- forceps x 1 - bottle x 1 - ants x 2  <u>Photos:</u> 
<b>Time</b>	29/4 (THU)
<b>Venue</b>	School Laboratory
<b>Photos</b>	<u>Procedure:</u> 1. Use the forceps to take out an ant out.
 	2. Place the ant near the edge of the pitcher plant.



3. Observe how the ants fall down.

**Investigate further: Dissection of pitcher plant**

Photos	Procedure
	<p>1. Choose one pitcher cup.</p>
	<p>2. Cut the tendril to separate the pitcher cup and the plant.</p>
	<p>3. Dissect the pitcher cup slowly.</p>



4. Observe the inner structure (digestive zone) of the pitcher cup under a microscope.

### Structure of the pitcher cup

Photos		Description
	<p>Cut the pitcher cup by using a pair of scissors.</p>	
	<p>The upper part of the pitcher has special peristome that make the insects slip into the cup easily.</p>	
	<p>For the lower part of the cup, the dotted with the digestive fluid trap the insects and they can hardly move and escape.</p>	

## Experiment 2: Enzyme Test

<b>Question</b>	How does pitcher plant digest insects?
<b>Aim of the experiment</b>	(a) To find out how pitcher plants capture insects. (b) The experiment focuses on the adaptive features for catching insects.
<b>Experimental design</b>	We assume that the pitcher fluid contains enzyme (protease). We use photographic films for testing since they have gelatine coat on the surface. If the pitcher fluid contains enzyme, the light-sensitive silver halide salts, which are black in colour, will be removed. It is because protease will digest the gelatine coat. And the film will become colourless. The result may show the presence of protease.
<b>Apparatus</b>	<ul style="list-style-type: none"> <li>- test tubes x 3</li> <li>- photographic films x 3 pieces</li> <li>- buffer solution pH 4</li> <li>- dish x 1</li> <li>- droppers x 2</li> <li>- stoppers x 3</li> <li>- pH paper x 3 pieces</li> </ul> <p><u>Photos:</u></p>   
<b>Date</b>	2/5 (THR) – 4/5 (SAT)
<b>Venue</b>	School laboratory
<p>◆ First, we test the pH value of the pitcher fluid. For an accurate result, we use two sets of</p>	

digestive fluid from two different pitcher plants. Then, we use the buffer solution, with the pH value same as the pitcher plant for the control set-up.

**Photos**



◆ **Procedure:**

1. Choose two pitcher plants randomly.



2. Transfer the fluid to the test tubes.



3. Place a piece of pH paper on the dish.



4. Add 1 – 2 drops of pitcher fluid on the pH paper.



**5. Results (pH value):**

Pitcher Plant 1 (left): pH 4

Pitcher Plant 2 (right): pH 4



6. Use a solution which is pH 4 to be the control set up.

7. Add 1- 2 drops of buffer solution (pH 4) on the pH paper.

**8. Results (pH value):**

Buffer solution: pH 4



9. Add a piece of photographic film to each test tube.



10. Place stoppers onto the mouth of the test tubes to prevent from drying out.

# Results of the experiments

## From Experiment 1:

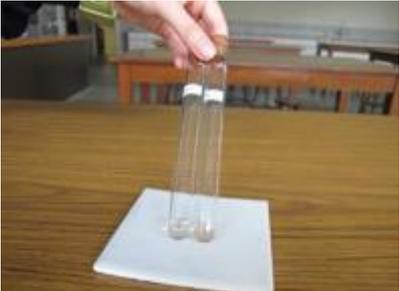
1 <sup>st</sup> ant		
Time	Photo	Observation
12:15pm		We put the bottle which contains an ant near the edge of the plant
12:15pm		After a few seconds, the ant slips down the pitcher cup.
12:17pm		The ant is trapped and by the pitcher fluid. And it is still struggling.
12:23pm		The ant cannot escape from the pitcher cup, and stop struggling.
12:32pm		The ant does not move anymore.

2<sup>nd</sup> ant

Time	Photo	Observation
12:40pm		<p>We use the forceps to take out an ant out. Then, place the ant on the edge of the pitcher plant.</p>
12:56pm		<p>After about 15 minutes, the ant slips down the cup.</p>
13:04pm		<p>The ant is struggling and keeps climbing on the wax plate to escape from the plant.</p>
13:09pm		<p>The ant cannot escape from the bottom of the plant.</p>

13:16pm		The ant does not move anymore.
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**From Experiment 2:**

Date	Result	Photo
Day 1 5:25pm 29/4 (Mon)	<p><b><u>The 1<sup>st</sup> observation</u></b></p> <p>All of them are colourless and without any observable changes</p>	<p>“fluid of pitcher plant 1” (right)            “fluid of pitcher plant 2” (centre)            Control set up (left)</p> 
Day 2 5:33pm 30/4 (Tue)	<p><b><u>The 2<sup>nd</sup> observation</u></b></p> <ul style="list-style-type: none"> <li>● The control set up (buffer solution) did not have any observable changes</li> <li>● The disc of film inside test tube “fluid of pitcher plant 1” became slightly turbid</li> <li>● The disc of film inside test tube “fluid of pitcher plant 2” became slightly turbid</li> </ul>	<p>Control set up            (buffer solution pH 4)</p>  <p>“fluid of pitcher plant 1” (right)            “fluid of pitcher plant 2” (left)</p> 

Day 3 5:30pm 1/5 (Wed)	<p><b><u>The 3<sup>rd</sup> observation</u></b></p> <ul style="list-style-type: none"> <li>● The control set up (buffer solution) did not have any observable changes</li> <li>● The fluid inside test tube “fluid of pitcher plant 1” became turbid and the colour of film changes from black to transparent ( become pale).</li> <li>● The fluid inside test tube “fluid of pitcher plant 1” became turbid and the colour of film changes from black to transparent (become pale).</li> </ul>	<p>“fluid of pitcher plant 1” (right)  “fluid of pitcher plant 2” (centre)  Control set up (left)</p> 
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From the result, we can prove the presence of protease in the pitcher fluid. It can explain why the pitcher plants can digest the insects after they fall into the pitcher cup.

## Discussion

### 1. Adaptive features of pitcher plant (Effective “eating” process)

From the results in Experiment 1, we concluded that the structures (e.g. special peristome, digestive fluid and the shape) of the pitch plants are the useful feature for trapping the insects. Physically, the special peristome surface is slippery for insects due to the concavo-convex surface, the shape and the wet wax plate of the cup will trap the insect and do not let them escape (*Proc Natl Acad Sci U S A. 2004 Sep 28*). Besides, the trapping efficiency of the fluid remains strong even when it is highly diluted by water (*PLoS One. 2007 Nov 21*).

Moreover, we focused on how the insect is trapped by the pitcher plant. By watching how the insect struggles in it and eventually dies, we know that the shape, the fluid and the peristome affect the most (*Proc Natl Acad Sci U S A. 2004 Sep 28*).

### 2. Protease in the pitcher fluid

From the results in Experiment 2, the presence of protease in the pitcher fluid is obvious. When the insects are slipped into the pitcher cup, it will secrete some digestive fluid which contains enzymes. The enzymes can break down the nutrients from the insects for absorption of the plants (*J Proteome Res. 2008 Feb*).

## Limitations of investigation

### 1. Not having a detailed investigation

Due to limited time, we could not carry out a detailed and intensive investigation on the features of pitcher plants. Pitcher plants may have other adaptive features to trap prey effectively, which we did not make an analysis on them. The project may not give people a clear studying about pitcher plants.

## **2. Insects we used for testing**

We used ants for testing how the insects would be trapped by the pitcher plant. The ants were found in the garden and maybe they are different kinds of ants. So the result may not be accurate.

## **3. Possibility of finding wild pitcher plants is low**

Because of the special living environment (they usually grow in swamps and marshes), it is difficult to find the pitcher plants in outdoor areas with suitable environment in Hong Kong. And its status in China is vulnerable, so it is certainly not easy to find them in the nature. Thus, we could not compare the wild pitcher plants and the pitcher plants bought in shops.

## **Conclusion**

After two experiments and the investigations, we found out the adaptive features (e.g. special peristome, digestive fluid and the shape) of the pitcher plants are important for them to "catch" their prey. Besides, we found out that there is enzyme in the digestive fluid to digest the insects. Both physical and chemical structures are useful and significant for their "hunting". All these results surprise each of us by their "special talents" and we finally realized the power of the nature. Such a green plant can be carnivorous, and as our title says, they are the "Butcher, Catcher, Pitcher Plants"!

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