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Plant carnivory in the Caryophyllales: phylogenetic relationships, morphological adaptations, and molecular evolution of digestive enzymes among carnivorous genera

by

Tanya Renner

A dissertation submitted in partial satisfaction of the
requirements for the degree of

Doctor of Philosophy

in

Plant Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Chelsea D. Specht, Chair

Professor Tom Bruns

Professor Rasmus Nielsen

Fall 2011

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Abstract

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Doctor of Philosophy in Plant Biology

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Professor Chelsea D. Specht, Chair

Phylogenetic relationships among carnivorous plants of the angiosperm order Caryophyllales are explored using Bayesian statistics and maximum-likelihood based searches of phylogeny. Nuclear ribosomal (ITS) and chloroplast intergenic spacer (PY-IGS) regions, along with previously-sequenced DNA are utilized for phylogenetic reconstructions. Taxonomic relationships across genera are refined and three strongly supported clades are identified: monophyletic Droseraceae, Nepenthaceae, and a third clade containing Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae. In combination with phylogenetic reconstruction, stochastic character mapping is utilized to assess evolutionary changes in the morphology of glands found on the lamina and involved in the digestion of prey. Adaptive changes resulting in the evolution of the carnivorous gland are discussed, which may have occurred either by emargination of the leaf blade or homologous transformation of pinnae.

A variety of enzymes are excreted from the carnivorous gland that aid in prey digestion. Within the carnivorous plants of the Caryophyllales, two subclasses of class I chitinases have been identified to play a role in the digestion of prey. Proteins produced by the large and diverse chitinase gene family are involved in the hydrolyzation of glycosidic bonds in chitin, a polymer of *N*-acetylglucosamines. Members of these subclasses, depending on the presence or absence of a C-terminal extension, can be secreted from specialized digestive glands found within morphologically diverse traps that develop from plant leaves. Homology among carnivorous plant class I chitinases and the method by which these enzymes have been adapted for the carnivorous habit are investigated. Novel class I chitinase homologs are recovered from *Ancistrocladus*, *Dionaea*, *Drosera*, *Nepenthes*, and *Triphyophyllum*, in addition to class I chitinases available from sequenced angiosperm genomes. Substitutions specific to carnivorous plant class I chitinases are revealed by detecting sites under positive selection, which may confer functional differences as indicated by protein structure homology-modeling.

To study gene function in non-model organisms, a virus induced gene silencing (VIGS) method was developed. VIGS has been shown to be effective for transient knockdown of gene expression in plants to analyze the effects of specific genes in development and stress related responses. It is demonstrated that the barley stripe mosaic virus (BSMV) is able to infect two species within the Zingiberaceae, and that BSMV-VIGS can be applied to specifically downregulate phytoene desaturase in the culinary ginger *Zingiber officinale*. BSMV-VIGS is likely to be effective in other angiosperms susceptible to BSMV infection. This should enable targeted studies for identifying gene function to be carried out in ecologically and evolutionarily important groups.

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In continuing with VIGS research, I began to plan other components of my dissertation project and proposed my topic to my advisor: the study of phylogenetic relationships among the carnivorous plants of the Caryophyllales and the molecular evolution of their digestive enzymes, specifically chitinase. Although independent from the lab's research on ginger floral morphology, Chelsea supported the individual nature of my research and allowed me to plow my own way through molecular biology. I applied for multiple grants to secure my stipend and research funding, all of which Chelsea provided a tremendous amount of feedback for. The research forming the basis of my dissertation was partially funded by UC Berkeley Committee on Research and the College of Natural Resources, a National Science Foundation Graduate Research Fellowship, a Chang-Lin Tien Graduate Fellowship in the Environmental Sciences, and a National Science Foundation Doctoral Dissertation Improvement Grant awarded to Chelsea Specht and myself (DEB 1011021). The skills I have learned from Chelsea to analyze and edit my scientific writings has been invaluable. Demonstratively, this skill set has already been applied to the chapters of my dissertation, the majority of which have already been accepted for publication. Throughout the development of these manuscripts, Chelsea consistently met with me to discuss methods and results, provide suggestions, and to mentor me through the rigors of journal article submission.

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Introduction

The Carnivorous Caryophyllales

The following dissertation research is focused on understanding the evolution of carnivory and chitinase genes in one of the major groups of plants that has evolved the carnivorous habit: the Caryophyllales. The greatest number of carnivorous plant families can be found in the Caryophyllales (Droseraceae, Drosophyllaceae, Nepenthaceae and Dioncophyllaceae) and within these families are the most numerous of carnivorous plants. Families are comprised of the carnivorous genera *Drosera* (sundews), *Dionaea* (Venus flytrap), *Aldrovanda* (aquatic flytrap), *Drosophyllum* (Portuguese sundew), *Nepenthes* (tropical pitcher plants), part-time carnivore *Triphyophyllum*, along with non-carnivorous genera *Ancistrocladus*, *Habropetalum*, and *Dioncophyllum*. Shared among the carnivorous plant lineage of the Caryophyllales is the presence of multicellular glands that have evolved to function in the secretion of digestive enzymes as well as to absorb amino acids and other organic nutrients.

Families sister to the carnivorous non-core Caryophyllales (Tamaricaceae, Frankeniaceae, Polygonaceae and Plumbaginaceae) have a variety of sessile, stalked, and pitted glands (Wilson 1890). Although rarely vascularized themselves, these glands occur near vascular tissue, are known to exude salt or mucilage and are described as use for protection in halophytic conditions, seed dispersal, and to deter herbivory (Fahn and Werker 1972; Faraday and Thomson 1986; Lüttge 1971; Sakai 1974; Wilson 1890).

In the genus *Drosera*, two basic types of carnivorous glands are present: (1) vascularized, stalked multicellular glands and (2) non-vascularized, sessile glands. The upper leaf surface of *Drosera* is saturated with both types of gland, while only type 2 is found on the abaxial side of the leaf as well as petioles and inflorescence scapes (Juniper et al. 1989). Although the glands co-exist on the leaf surface, each gland type has different cellular origins. Cells of type 1 are either epidermal or parenchymatous in origin with tracheids and xylem embedded within, which extend into the veins of the leaves (Gilchrist and Juniper 1974). Conversely, type 2 are exclusively epidermal in origin and lack vasculature at maturity (Juniper et al. 1989).

In the monotypic genus *Dionaea* (*D. muscipula*) multiple types of sessile glands are present, all of which are non-vascularized. Sessile glands differ in their morphology depending whether the glands are located externally (abaxially) or internally (adaxially) on the plant trap. Glands found on the abaxial side of the leaf are stellate, whereas glands on the adaxial side (those involved in digestion) are made up of a variety of cell types which include a single basal cell, a stalk cell, and 8 or more digestive gland cells (Scala et al. 1968). In addition to the sessile glands on the digestive surface of the trap, trigger hairs are located at the very edge of the trap where the adaxial and abaxial sides meet. These structures are vascularized and are homologous in their position to the marginally located vascularized, stalked multicellular glands in *Drosera*.

Aldrovanda (*A. vesiculosa*) is an aquatic relative of *D. muscipula* and the only extant member of its genus. Each vegetative node contains eight leaves arranged in a whorl evenly distributed around the stem, with the carnivorous trap (the leaf blade) attached by a petiole to the leaf base. Similar to *Dionaea*, multiple types of non-vascularized, sessile glands can be found on the surface of the trap. Ashida divided the trap of *A. vesiculosa* into 2 morphological zones: marginal and central (Ashida 1935). The marginal zone consists of the rim, quadrifid, and hairless regions of the trap. The rim, as in *Dionaea*, has teeth where the adaxial and abaxial sides of the leaf meet. These are hypothesized to be reduced stalked glands and may retain vestiges of vasculature. The quadrifid region has two

types of glands: quadrifid (4-lobed) glands on the adaxial surface and 2-lobed glands on the abaxial surface. The hairless region, which is not entirely hairless or glandless, has only 2-armed glands on the adaxial side of the leaf. The glands of the marginal zone may all be modified forms of sessile glands not involved in digestion of prey.

The central zone consists of the digestive, midrib, and detention regions, all of which have sessile glands that function in digestion of prey. The digestive region contains numerous small sessile glands on the adaxial surface, 2-armed glands on the abaxial surface, and >15 trigger hairs on each lobe. The midrib region also has trigger hairs (>10) as well as sessile glands on the adaxial surface. The detention region has sessile glands on the adaxial surface, but are not as numerous as those found in the digestive region of the adaxial midrib region.

Drosophyllum contains the single species *Drosophyllum lusitanicum*, a carnivorous plant with a shrub-like habit that lives among dry, alkaline soils (Harshberger 1925). The leaves of *D. lusitanicum* are linear with a major groove on the adaxial side of the blade. Unlike in *Drosera*, *D. lusitanicum* leaves show reverse circinate vernation; a character that is shared with *Triphyophyllum peltatum*. Attached to these leaves are both stalked and sessile glands that are accompanied by a network of vascular bundles. The stalked and sessile glands are organized by having a layer of cuticle, two layers of stalk cells and finally, a central vessel of xylem and phloem (Juniper et al. 1989).

Only a single member of Dioncophyllaceae, *Triphyophyllum peltatum*, is considered carnivorous, albeit only a part-time carnivore. *T. peltatum* exhibits seasonal heterophylly and is able to produce three types of leaves throughout its growth: conventional photosynthetic, carnivorous, and tendriled (Juniper et al. 1989; Shaw 1951; Sprague 1916). Carnivorous leaves last only a few weeks and are found on young growth. They are hypothesized to enable the plant to trap and digest insects to provide supplemental nourishment before the plant bolts into a wild liana (Bringmann et al. 2002). Field observations have noted that carnivorous leaves also arise when the plant has been injured (Metcalf 1951) or on offshoots (McPherson 2008). While not in its carnivorous stage, *T. peltatum* produces tendriled and conventional photosynthetic leaves similar that of *Dioncophyllum tholloni* and *Habropetalum dawei*: this includes leaves on long shoots that bifurcate at the laminal apex into tendril hooks for climbing, and leaves on short shoots without tendril hooks (Sprague 1916).

The carnivorous leaves of *T. peltatum* have stalked and sessile multicellular glands that are vascularized with xylem and phloem (Metcalf 1951) and share morphological characteristics at the cellular level. Both stalked and sessile glands are composed of two to four layers of outer secretory cells over domed endodermal cells. Within the endodermal layer, groups of branched vascular tracheids can be found ensheathed in parenchyma with numerous amyloplasts (Juniper et al. 1989). The glands are connected to the central vascular system of the midrib, with vascular traces leading from the stalked glands being wider in diameter than those that vascularize the sessile glands (Metcalf 1951).

The glands of *T. peltatum* carnivorous leaves show a high degree of affinity to Drosophyllaceae. The stalked glands are similar in structure to those of *D. lusitanicum* (Green et al. 1979), containing vascular tissue that includes both xylem and phloem (unlike Droseraceae). The stalked glands of *T. peltatum* are immobile and are arranged on filiform leaves that show reverse circinate vernation, similar to those of *D. lusitanicum* (Green et al. 1979). This differs from *Drosera*, in which stalked glands can be highly mobile and are arranged on leaves that have circinate vernation but are not always filiform. In *D. tholloni* and *H. dawei*, multicellular stalked and sessile glands are absent from the leaves. However, it should be noted that numerous glandular hairs with flattened multicellular heads can be found on the stems of *H. dawei* (Metcalf 1951).

Ancistrocladus is a genus of non-carnivorous wild lianas that contains ~20 species and can be found in Africa and Asia (Cheek 2000; Foster and Sork 1997; Gereau 1997; Taylor et al. 2005). Similar to *D. tholloni* and *H. dawei*, *Ancistrocladus* is a wild liana and hooks are formed from modified stem apices for climbing. On the abaxial side of the leaf, glandular pits partially covered by the epidermis can be found. These glands are thought to function in wax secretion and are similar in their morphological structure to the pitted glands of Nepenthaceae (Metcalf 1951; Taylor et al. 2005). Given our current phylogeny, these wax-secreting glands may be an ancestral character that was lost in Droseraceae.

The genus *Nepenthes* comprises ~120 species found primarily in South East Asia with its center of diversity in the islands of Indonesia (Meimberg and Heubl 2006). Inside the pitcher, a variety of zones involved in trapping insects are found. Near the entrance to the pitcher and along the peristome, extrafloral nectaries are present. These nectaries are sunken, partially covered by epidermis, and surrounded at their periphery with bundles of phloem (Owen et al. 1999). The upper one-third of the pitcher is a waxy zone that, while not considered essential for trapping prey (Gaume 2009), can aid in trapping by providing a slick surface that is difficult for insect prey to climb (Knoll 1914). At the base of the pitcher are glands that are involved in digestion. Similar in morphology to the extrafloral nectaries, these glands are also partially covered by epidermis (Parkes 1980). Closer to the waxy zone of the pitcher, the epidermal overhang or epidermal ridge of these glands covers the majority of the glandular pit. Yet toward the base of the pitcher, these overhangs are almost completely absent. Gland structure is defined by one or more layers of secretory and endodermal cells overlaid with columnar cells and a cuticle (Owen and Lennon 1999). Often tracheids are found near these endodermal cells, although the glands themselves do not contain xylem and phloem (Lloyd et al. 1942; Rottloff et al. 2009).

The study detailed in chapter 1 investigates the morphological evolution of glands involved in the digestion of prey. Currently unresolved phylogenetic relationships are refined across carnivorous genera of the non-core Caryophyllales and the phylogeny is used to investigate the morphological evolution of glands involved in the digestion of prey, via stochastic character mapping (Renner and Specht 2011). Ancestral gland types are identified and it is tested whether certain types of glands are consistent indicators of plant carnivory. The phylogenetic results also detail gain and loss of plant carnivory in the Caryophyllales.

Chitinolytic Enzymes of the Carnivorous Caryophyllales

The isolation and characterization of carnivorous plant digestive enzymes secreted from glands began in the nineteenth century, when Sir Joseph D. Hooker discovered the first protease in *Nepenthes* sp. trap fluid cir. 1874 (Lönning and Becker 2004). One year later, Charles R. Darwin published his accounts on *Drosera rotundifolia* and the ability of *Drosera* to digest nitrogenous and phosphate-containing compounds (Darwin 1875). However, it was not until the 1970s that the basic enzyme composition in carnivorous plant mucilage was characterized on a global scale. Among the various enzymes identified to be important in plant carnivory, chitinases have been one of the most thoroughly studied (Amagase et al. 1972; Robins and Juniper 1995; Matusíková et al. 2005; Eilenberg et al. 2006; Hatano and Hamada 2008).

Plant chitinases (EC 3.2.1.14) commonly act as pathogenesis-related (PR) proteins, which are either induced in response to insect herbivory and fungal elicitors, or constitutively expressed in tissues vulnerable to attack (Brogue et al. 1988; Samac et al. 1990). Chitinases belong to either

glycoside hydrolase (GH) families 18 or 19 and function in the hydrolysis of β -1,4-glycosidic bonds between *N*-acetylglucosamine (NAG) oligomers residues in chitin polymers (Legrand et al. 1987). GH families 18 and 19 chitinases have been identified in all plants analyzed to date, many of which have been shown to inhibit fungal growth in vitro and enhance resistance to fungal pathogens in transgenic plants (Schlumbaum et al. 1986; Brogue et al. 1988; Leah et al. 1991; Jach et al. 1995; Eilenberg et al. 2006). GH families 18 and 19 do not share sequence similarity or three-dimensional structure eventhough these enzymes perform similar functions, which may suggest that chitinases arose independently from one another (Hamel et al. 1997).

Plant chitinases are encoded by large gene families, which have been previously organized into five classes according to sequence, structure and phylogenetic relationship. Class I, II, and IV chitinases belong to glycoside hydrolase (GH) family 19, and share a homologous catalytic domain as well a signal peptide at the amino terminus (Collinge et al. 1993; Araki and Torikata 1995; Neuhaus et al. 1996; Hamel et al. 1997). Class I is divided into two subclasses, each with a highly conserved cysteine-rich region involved in chitin-binding (Beintema 1994; Araki and Torikata 1995). Subclass Ia has a carboxyl terminal extension (CTE) that codes for transmission to the vacuole, while subclass Ib is extracellular due to the absence of a CTE (Neuhaus et al. 1995). Class IV comprises extracellular chitinases with similar domain architecture to class I, but which are significantly smaller due to deletions within the chitin-binding and catalytic domains (Passarinho and de Vries 2002). Class II chitinases lack the cysteine-rich region and CTE, but the catalytic domain may be very similar in sequence to class I chitinases (Araki and Torikata 1995). Class III and V chitinase are placed in GH family 18, are more similar to fungal and bacterial chitinases than to other plant chitinases, and have been found to exhibit additional lysozyme activities (Majeau et al. 1990; Graham and Sticklen 1994; Heitz et al. 1994).

Active production of chitinolytic enzymes were first demonstrated in *Drosera* and *Nepenthes* by Amagase et al. (1979), demonstrating digestion of colloidal chitin to increase over time in the presence of concentrated trap secretions. Several years later, Robins and Juniper (1995) found *Dionaea muscipula* traps to exhibit chitinase activity similar to that in *Drosera* and *Nepenthes*. However, none of these studies could rule out the possibility that a symbiotic microorganism could have created this chitinolytic activity. It was not until 2006 that these chitinases were shown to be endogenous to the plant itself. A study using sterile *Nepenthes khasiana* pitchers revealed two subclasses of class I chitinases (Ia and Ib) to be present in the secretory region of the trap (Eilenberg et al. 2006). Interestingly, each subclass had differential expression; subclass Ia being constitutively expressed and subclass Ib found to be upregulated in response to colloidal chitin. During the same year, Matusíková et al. (2005) demonstrated the localized expression of class I chitinase within the tentacles of *Drosera rotundifolia* via *in situ* hybridization after induction with chitin. For subclass Ib, the absence of a CTE is hypothesized to allow excretion from the secretory cells and thus make them available in the trap for use in plant carnivory (Eilenberg et al. 2006).

Interestingly, in non-carnivorous plants studied, class I chitinases have been identified to be under strong positive selection. Bishop et al. (2000) found that adaptive replacements occur disproportionately in the active site cleft in *Arabidopsis* spp. subclass Ia chitinases. Further studies of subclass Ia chitinases in Poaceae (Tiffen 2004) have found similar replacements within the same sites. These observations are proposed to be due to an evolutionary arms race between the chitinolytic enzymes and competitive inhibitors produced by fungal pathogens. Could having a method (an absence of a CTE) by which to escape the digestive gland allowed for subfunctionalization of subclass Ia chitinases for pathogen response and subclass Ib chitinases for plant carnivory? In addition, if carnivorous plant subclass Ib chitinases are used primarily for the

prey digestion and not for pathogenic response against fungi, have subclass Ib enzymes been released from positive selection to conserve amino acid structure at the active cleft? The first molecular evolutionary studies of class I chitinases in the carnivorous plants of the Caryophyllales attempt to address these questions in chapter 2.

A method for studying gene function in non-model organisms

Virus-induced gene silencing (VIGS) is a technique that utilizes the RNA interference (RNAi) pathway to downregulate endogenous gene expression (Dinesh-Kumar et al. 2003; Burch-Smith et al. 2004; Godge et al. 2008). The experimental procedure is in most instances rapid, making it more desirable as an application comparably to *de novo* production of knockout mutants or analyses of transformed RNAi plants.

Susceptibility to viral infection is a critical component of VIGS. A variety of viruses have been utilized to down-regulate gene expression in model plants: tobacco mosaic virus (TMV) in *Nicotiana tabacum* L. (Kumagai et al. 1995), potato virus X (PVX-VIGS) in wild-type *Nicotiana benthamiana* Domin (Ruiz et al. 1998), tobacco rattle virus (TRV) in members of the Solanaceae and Brassicaceae (Ratcliff et al. 2001; Burch-Smith et al. 2004; Chen et al. 2004; Fu et al. 2005; Burch-Smith et al. 2006; Dong et al. 2007; Godge et al. 2008), and pea early browning virus (PEBV) in legumes (Constantin et al. 2004, 2008). Among the cereal crops, barley stripe mosaic virus (BSMV-VIGS) has been utilized to induce VIGS in barley (*Hordeum vulgare* L.) (Holzberg et al. 2002; Bruun-Rasmussen et al. 2007) and wheat (*Triticum aestivum* L.) (Scofield et al. 2005), but prior to the work detailed in chapter 3, application of VIGS to monocots other than cereal grasses had not been described.

After establishing that BSMV caused systemic infections in the Zingiberales, BSMV-VIGS was applied to *Zingiber officinale* using a combination of three BSMV transcripts consisting of RNA α , RNA β derivative (B7) that is deficient in expression of the coat protein (CP), and RNA γ modified to block the expression of the γ b VIGS suppressor and to enable the insertion of *Z. officinale* phytoene desaturase (PDS) cDNA (Petty et al. 1990; Bragg and Jackson 2004). PDS is an excellent gene to assay VIGS as it encodes for an enzymes involved in the biosynthesis of carotenoids. Once silenced, PDS is unable to protect chlorophyll from photo-oxidation, resulting photobleaching due to decreased carotene content (Benedito et al. 2004). In addition to the observed phenotypes resulting from PDS downregulation, decreased levels of phytoene desaturase RNA were evident following semi-quantitative PCR. These results indicate that BSMV can efficiently downregulate expression of specific target genes in *Z. officinale*.

The particular VIGS technique described in chapter 3 was developed out of interest in studying gene function in non-model plants. Importantly, demonstrated is the use of a non-native virus to induce VIGS in a phylogenetically distant taxon (Renner et al. 2009). These results suggest that the VIGS technique can be used among a variety of species, dependent on viral susceptibility. If a suitable virus can be identified, there is a great potential for the technique to be extended to future characterizations of digestive enzyme function in the carnivorous plants of the Caryophyllales.

Chapter One

A sticky situation: assessing adaptations for plant carnivory in the Caryophyllales utilizing stochastic character mapping

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Abstract

Phylogenetic relationships among carnivorous plants of the angiosperm order Caryophyllales have been explored, although a robust phylogeny encompassing all carnivorous genera is absent. We sample nuclear ribosomal (ITS) and chloroplast intergenic spacer (PY-IGS), along with previously-sequenced DNA from members of the non-core Caryophyllales for use in Bayesian statistics and maximum-likelihood based searches of phylogeny. Taxonomic relationships across genera are refined and three strongly supported clades are identified: monophyletic Droseraceae, Nepenthaceae, and a third clade containing Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae. In combination with phylogenetic reconstruction, stochastic character mapping is utilized to assess evolutionary changes in the morphology of glands found on the lamina and involved in the digestion of prey. The presence of sessile glands is identified as the likely ancestral character state and stalked and pitted glands are suggested to have been acquired independently by ingroup and outgroup taxa. Additionally, in some genera we found a lack of association between gland vasculature and plant carnivory, demonstrating that internal architecture of glands is not indicative of whether the plant is a functional carnivore. Finally, we discuss how adaptive changes resulting in the evolution of the carnivorous gland may have occurred either by emargination of the leaf blade or homologous transformation of pinnae.

1.1 Introduction

Phylogenetic relationships among the carnivorous genera of the Caryophyllales

Léon Croizat once proposed that all carnivorous plants comprised a single lineage based upon similarities in trap type, and that carnivory represented an early condition of the Angiosperms (Croizat 1960). More recently, however, carnivory has been shown to be a derived condition and is hypothesized to have arisen independently at least five times within angiosperms, in angiosperm orders Ericales, Lamiales, Oxalidales, Poales, and Caryophyllales (APGII 2003), suggesting convergent evolution of the carnivorous habit across angiosperms. The greatest number of carnivorous plant species are found in the non-core Caryophyllales (Cuénoud et al. 2002) in families Droseraceae (*Aldrovanda*, *Dionaea*, *Drosera*), Drosophyllaceae (*Drosophyllum*), Nepenthaceae

(*Nepenthes*) and Dioncophyllaceae (*Triphyophyllum*) (Rice 2006). The carnivorous plant lineage of the Caryophyllales is also unique in that it appears as though plant carnivory arose once and was subsequently lost by closely-related members of Ancistrocladaceae (*Ancistrocladus*) and Dioncophyllaceae (*Dioncophyllum* and *Habropetalum*) (Heubl et al. 2006).

Previous phylogenetic analysis of the carnivorous Caryophyllales have focused sampling on species within Droseraceae (Rivadavia et al. 2003), Nepenthaceae and Ancistrocladaceae (Heubl et al. 2006; Meimberg et al. 2000, Meimberg and Heubl 2006, Meimberg et al. 2006) independently or have limited sampling designed to test the placement of *Dionaea* and *Aldrovanda* (Cameron et al. 2002). Gene regions used in these analyses tend to be either slowly evolving markers (*rbcL*, *matK*, *atpB*) selected to investigate backbone relationships, or more rapidly evolving markers (18S, *trnK*, PTR1) selected to investigate species-level relationships within a given family or genus. In the current study, we add the chloroplast intergenic spacer between *psaA* and *ycf3* genes (PY-IGS) and nuclear ribosomal DNA (ITS) to molecular data previously published for analyses of phylogenetic relationships among the carnivorous plants of the Caryophyllales (*atpB*, *matK*, *petB*, PTR1, *rbcL*, *trnK*). We sample all carnivorous genera, including multiple representatives of each genus where possible in order to test phylogenetic relationships among genera.

Glands involved in plant carnivory in the Caryophyllales

The non-core Caryophyllales share a number of synapomorphies, including the possession of pitted, sessile, and stalked glands (Judd et al. 2002). In Plumbaginaceae and Polygonaceae, families sister to the carnivorous plants of the Caryophyllales, glands are rarely vascularized and function in the secretion of salt in halophytic conditions, in the secretion of mucilage to deter herbivory, or for dispersal (i.e. epizoochory) (Fahn and Werker 1972; Faraday and Thomson, 1986; Lüttge 1971; Sakai 1974). In carnivorous genera, homologous glands have apparently evolved to function in the secretion of digestive enzymes and to absorb amino acids and other organic nutrients (Amagase 1972; Eilenberg et al. 2006; Hatano and Hamada 2008; Juniper et al. 1989; Morrissey 1964; Owen et al. 1999). Of the three gland types that function in plant carnivory, sessile and stalked glands have diversified extensively to include vascularized forms.

Members of Droseraceae include the sundews (genus *Drosera*), in addition to the monotypic Venus Flytrap (*Dionaea muscipula*) and its aquatic sister species *Aldrovanda vesiculosa* (Cameron et al. 2002; Rivadavia et al. 2003). Species of Droseraceae can either have sessile glands or a combination of sessile and stalked glands. In *Drosera*, two basic types of carnivorous glands are present: (1) vascularized, stalked glands and (2) non-vascularized, sessile glands. The upper leaf surface of *Drosera* is densely covered with both types of gland, while only type 2 is found on the abaxial side of the leaf, petioles, and inflorescence scapes (Juniper et al. 1989). Cells of type 1 are either epidermal or parenchymatous in origin with tracheids and xylem embedded within, which extend into the veins of the leaves (Gilchrist and Juniper 1974). Conversely, the cells of type 2 are exclusively epidermal in origin and lack vasculature at maturity (Juniper et al. 1989). In *D. muscipula*, non-vascularized, sessile glands are located abaxially or adaxially on the plant trap. Glands found on the abaxial side of the leaf are stellate, whereas glands on the adaxial side (those involved in digestion) are made up of a variety of cell types, which include a single basal cell, a stalk cell, and 8 or more digestive gland cells (Scala et al. 1968). In addition to the sessile glands on the digestive surface of the trap, trigger hairs can be found adaxially and centrally on each lobe of the lamina (Juniper et al. 1989). Interlocking vascularized ‘teeth’ are present at the leaf margin and are homologous in their position to the

marginally located stalked glands in *Drosera* (Juniper et al. 1989). In the sister species *A. vesiculosa*, non-vascularized, sessile glands are present in each trap and may be either 4-lobed when placed adaxially or 2-lobed abaxially (Ashida 1935). Similarly to *D. muscipula*, *A. vesiculosa* traps have teeth at the margin of the leaf.

The genus *Drosophyllum* contains the single species *D. lusitanicum*, a carnivorous plant with a shrub-like habit that lives among dry, alkaline soils (Harshberger 1925). Attached to the leaves of *D. lusitanicum*, are both stalked and sessile glands accompanied by a network of vascular bundles (Juniper et al. 1989). *Drosophyllum* is currently placed in its own family, Drosophyllaceae, with reported affinities to the Dioncophyllaceae (see discussion). *Triphyophyllum peltatum* (Dioncophyllaceae), a plant considered to be carnivorous during some periods of its development, has sessile and stalked glands similar in architecture to those of *D. lusitanicum* – being filled with a central vessel of xylem and phloem (Green et al. 1979). Interestingly, *Dioncophyllum tholloni*, a close sister taxon to *T. peltatum* (fig. 1.1), is not considered carnivorous and does not display glands on its lamina.

Closely related to Dioncophyllaceae is the genus Ancistrocladaceae, a family of non-carnivorous lianas from Africa and Asia (Cheek 2000; Foster and Sork 1997; Gereau 1997; Taylor et al. 2005). In members of Ancistrocladaceae, glandular pits that function in wax secretion can be found on the abaxial side of the lamina (Taylor et al. 2005). Although not vascularized, Ancistrocladaceae glands are approached by vasculature that terminates abruptly below the pitted glands (Metcalfe 1951).

In *Nepenthes* (Nepenthaceae), a genus of ~130 species native to tropical Asia (McPherson 2010), pitted glands partially covered by the epidermis are located at the base of a modified lamina (the pitcher), which acts as the carnivorous trap. Gland structure is defined by one or more layers of secretory and endodermal cells overlaid with columnar cells and a cuticle (Owen et al. 1999). Often tracheids are found near these endodermal cells, although the glands themselves do not contain xylem and phloem (Lloyd 1942; Rottloff et al. 2009).

Although the glands present in the carnivorous Caryophyllales have been described previously in great detail, assessments of gland homology have not resulted in a unanimous conclusion as to their developmental origin. In more recent papers and reviews, digestive glands have been considered homologous with trichomes (Heubl et al. 2006), hairs (Chase et al. 2009), or epidermal cells (Owen and Lennon 1999). Defining the carnivorous gland may be confounded by differences in gland morphology between genera. In order to investigate the evolution of gland morphology, we explore the morphology of glands among carnivorous genera utilizing stochastic character mapping to further understand how carnivorous glands may have evolved in the Caryophyllales.

The aims of the present study are to (1) evaluate past phylogenetic analyses which included carnivorous taxa of the Caryophyllales, (2) determine the utility of PY-IGS and ITS molecular markers for phylogenetic reconstruction at the level of genus and species in the Caryophyllales, and (3) investigate gland morphology among carnivorous taxa and closely related non-carnivorous taxa under a phylogenetic framework in order to identify characteristics that are key in determining whether glands found on the lamina of the leaf are indicative of plant carnivory.

1.2 Material and Methods

Taxon sampling and tissue collection

A total of 51 taxa from families Ancistrocladaceae, Dioncophyllaceae, Droseraceae, Drosophyllaceae, Plumbaginaceae, Nepenthaceae, and Polygonaceae were included in our analyses (appendix). Taxa were selected to represent biogeographic diversity across the carnivorous Caryophyllales. Samples collected originated mainly from living collections at the University of California Botanical Garden, Missouri Botanical Garden, Universität Würzburg and California Carnivores in Sebastopol, California. Freshly collected leaf tissue was preserved in silica gel, frozen at -80°C, and vouchers were deposited at the University of California Herbarium. *Dioncophyllum tholloni*, a species not easily obtained from living collections, was collected in the wild by Gretchen Walters (MO), vouchered, and tissue was sampled from the herbarium sheet. We were unable to obtain tissue for *Habropetalum dawei* (Dioncophyllaceae).

Molecular marker sampling

The nuclear ribosomal DNA including internal transcribed spacer (ITS) and the chloroplast intergenic spacer between *psaA* and *ycf3* genes (PY-IGS) were chosen for phylogenetic reconstruction based on previous studies demonstrating the utility of these markers for resolution at both genus and species levels within the eudicots and Nepenthaceae (Alejandro et al. 2008; Downie et al. 1996; Meimberg 2002; Miranda et al. 2010; Sang et al. 1997; Shi et al. 2001; Tan et al. 2002; Tate et al. 2003). A previous study attempted to utilize ITS in Droseraceae, yet with unsatisfactory results (Miranda et al. 2010). Total genomic DNA was extracted from leaf base (*Aldrovanda* and *Dionaea*), leaf lamina (*Ancistrocladus*, *Dioncophyllum*, *Drosera* and *Drosophyllum*) and the lamina-like region of the leaf-base (*Nepenthes* and *Triphyophyllum*) using a cetyltrimethyl ammonium bromide (CTAB) (Doyle and Doyle 1987) or modified sodium dodecyl sulfate (SDS) and sodium chloride protocol (Edwards et al. 1991). Extracted genomic DNA was quantified using a NanoDrop 1000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA).

Previously published sequences for *atpB*, *matK*, *petB*, PTR1, *rbcL*, *trnK* were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>) for phylogenetic analyses (appendix). Of these molecular markers, only *matK* was available for *H. dawei* (GenBank: AF204845). In order to minimize missing data, outgroup genera *Limonium* (Plumbaginaceae) and *Polygonum* (Polygonaceae) are formed from composite sampling of *atpB*, ITS, *matK*, *petB*, PTR1, PY-IGS, *rbcL*, *trnK* sequences from multiple species where necessary. Taxa utilized in our analyses are summarized in A.1, along with voucher information and the GenBank accession number for each DNA sequence.

PCR and DNA sequencing

Previously published primer pairs ITS5a (Downie et al. 1996) and ITS4 (White, 1990) were used to amplify ITS in 10 µl aliquots with 10-100 ng of genomic DNA and the following reagents: 0.02 U iProof™ Polymerase (Bio-Rad, Hercules, CA, USA), 1x HF iProof buffer (Bio-Rad), 2.0 mM MgCl₂, 0.2 mM each of dNTP, 0.5 µM of each primer, 0.025 mg/mL BSA and 100% DMSO. PCR reactions were run on a MyCycler (Bio-Rad) thermal cycler under the following conditions: an initial denaturation for 3 min at 98°C, followed by 30 cycles of 98°C for 10 s, 54°C for 25 s, 72°C

for 30 s (increasing 0.03 degrees with each cycle), and ending with a final extension at 72°C for 7 minutes. For PY-IGS, PG1f and PG2r (Tan et al. 2002) were used to amplify PY-IGS from the majority of species using the same PCR chemistry as described for ITS, but with the following thermal cycler conditions: 5 min at 98°C, followed by 40 cycles of 98°C for 10 s, 60°C for 30 s, 72°C for 30 s, and ending with a final extension at 72°C for 7 minutes. For *D. lusitanicum* and *D. tholloni*, PY-IGS was amplified from using newly designed primers PFF592 (5'-CAG-TCA-AGT-AAT-TAG-TGA-ACC-3') and PFF593 (5'-AGT-TAT-TCA-CTC-GAA-CAA-TTA-3') using the Phire® Plant Direct PCR Kit and the suggested 3-step PCR protocol with annealing temperature set at 50°C (Finnzymes Inc., Woburn, MA, USA).

Prior to sequencing, PCR products were purified using exonuclease I and shrimp alkaline phosphatase to remove single-stranded primers and remaining dNTPs (Fermentas International Inc., Burlington, Ontario, Canada). PCR products were cycle sequenced using PCR primers and the ABI Prism BigDye Terminator Cycle Sequence Ready Reaction Kit v3.1 (Perkin-Elmer/Applied Biosystems, Foster City, CA, USA). Products of cycle sequencing were resolved on an ABI Prism 3100 automated sequencer (Applied Biosystems, Foster City, CA, USA).

Alignment and phylogenetic analyses

Forward and reverse sequences for ITS and PY-IGS were assembled and edited with Sequencher v.4.7 (Gene Codes Corp.). A multiple sequence alignment for 19 taxa (appendix) was constructed from ITS, PY-IGS, *atpB*, *matK*, *petB*, PTR1, *rbcl*, and *trnK* with ClustalX under default settings (Thompson et al. 1994) with subsequent manual adjustment in Mesquite v2.72 (Maddison and Maddison 2010). We excluded regions from ITS, PY-IGS, *atpB*, *matK*, *petB*, PTR1, *rbcl*, *trnK* alignments that were poorly aligned across the entire dataset, and combined all sequences into a single concatenated dataset (hereafter known as the combined dataset) with a final length of 9988 bp (A.2).

Bayesian inference (BI) of phylogeny was conducted in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) utilizing the combined dataset and partitioned for each molecular marker under the best-fit model of evolution as determined by the Akaike information criterion (AIC) in jModelTest v0.1.1 (Posada 2008): GTR (PTR1), GTR+G (*atpB*, *matK*, *petB*, *trnK*) or GTR+G+I (ITS, PY-IGS, *rbcl*). Two Bayesian analyses were performed simultaneously with posterior probabilities of the generated trees approximated using the Metropolis-coupled Markov chain Monte Carlo algorithm with four incrementally heated chains for 22000 generations while sampling trees every 100 generations until both analyses converged on similar log likelihood scores (average standard deviation of split frequencies <0.01). The first 110 trees were discarded as burn-in and a 50% majority rule tree was assembled from the remaining trees. Our Bayesian analyses including *matK* from *H. dawei* were unable to converge; most likely an artifact of increased missing data. Therefore, molecular data for *H. dawei* was removed from our phylogenetic analyses.

Maximum likelihood (ML) searches were performed using the interactive GARLI OSX GUI interface (Zwickl 2006) with an unpartitioned version of the combined dataset for 1000 bootstrap replicates. A consensus of bootstrap trees was constructed with SumTrees v3.0.0 using the DendroPy Phylogenetic Computing Library v3.7.1 (Sukumaran and Holder 2010). Trees were edited in Mesquite v2.72 (Maddison and Maddison 2010) and Adobe® Illustrator®.

Two Bayesian analyses of a dataset containing ITS and PY-IGS for 51 taxa (A.3) were performed in order to compare tree topology with the combined dataset of eight nDNA, nrDNA,

and cpDNA molecular markers. These analyses were made simultaneously in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) with GTR+G+I as determined by AIC in jModelTest v0.1.1 (Posada 2008). Posterior probabilities of the generated trees were approximated using the MCMC algorithm with methods similar to the combined eight molecular marker dataset. The first 2500 trees were discarded as burn-in and a 50% majority rule tree was assembled from the remaining trees as unrooted.

Character state reconstructions

Ancestral character reconstructions were conducted using maximum parsimony (MP) in MacClade v4.08 OSX (Maddison and Maddison 2005), ML in Mesquite v2.72 (Maddison and Maddison 2010), and Bayesian stochastic character mapping (Huelsenbeck et al. 2003) in SIMMAP v1.5 (Bollback 2006). Character reconstruction results were consistent among these different methods, therefore we only describe the Bayesian approach here.

We utilized the 50% majority rule tree generated in our BI analyses for the combined dataset to create stochastic mappings of gland types found on the lamina of the leaf and vasculature tissues associated with glands in SIMMAP. To assess evolutionary changes in gland morphology specifically related to plant carnivory, we scored character states as unordered for 3 gland types, taking in account the types of vasculature that can be found in each: (1) sessile glands; 0 = absence, 1 = presence, 2 = xylem and phloem (2) stalked glands; 0 = absence, 1 = presence, 2 = xylem, 3 = xylem and phloem (3) pitted glands; 0 = absence, 1 = presence. Descriptions of genera and illustrations of micrographs were used to determine character states and a summary of basic gland types and their morphologies (appendix). We chose to set bias and rate parameters with priors determined by a MCMC configuration calculated in SIMMAP for each gland type. For sessile glands, the bias parameter was set to equal (1/k) and we used a gamma rate prior with shape parameters $\alpha = 1.062$ and $\beta = 0.049$. For stalked glands, the bias parameter was similarly set to equal (1/k) and a gamma rate prior with shape parameters $\alpha = 0.671$ and $\beta = 0.011$ was used. Lastly, for pitted glands, $\alpha = 5.946$ was set as the beta distribution prior for the bias parameter and we used a gamma rate prior with shape parameters $\alpha = 0.972$ and $\beta = 0.019$. Default values for the number of categories (κ) for both beta and gamma distributions were used. Ancestral states at each node were calculated as the marginal posterior probability of each possible character state, which is dependent on the branch lengths and topology of the phylogenetic tree given (fig. 1.3A-C).

1.3 Results

Phylogenetic reconstruction

ML and BI methods of phylogenetic reconstruction gave congruent topologies that support monophyly of the carnivorous plant families of the Caryophyllales. Three strongly supported clades were identified corresponding with (a) a monophyletic Droseraceae, (b) a monophyletic Nepenthaceae, and (c) a third clade containing members of Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae (fig. 1.1, 1.2).

In ML and BI reconstructions of phylogeny for the combined dataset (fig. 1.1), Nepenthaceae (100 bs/1.00 pp) is placed as sister to the clade containing members of Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae with relatively strong support (89 bootstrap support (bs)

value/1.00 posterior probability (pp) value). The monotypic *Drosophyllum* is supported (100 bs/1.00 pp) as sister to a clade containing Dioncophyllaceae and Ancistrocladaceae. Within the Dioncophyllaceae, *Dioncophyllum* and *Triphyophyllum* are sister (1.00 bs/1.00 pp) and together are sister to a clade containing all included members of the genus *Ancistrocladus* (Ancistrocladaceae; 100 bs/1.00 pp), with high support (100 bs/1.00 pp). Droseraceae is recovered as monophyletic (97 bs/1.00 pp), with *Dionaea* and *Aldrovanda* sister to members of *Drosera*. Lastly, *Dionaea* and *Aldrovanda* are sister to one another with high support (100 bs/1.00 pp).

To determine if tree topology for the carnivorous Caryophyllales is conserved among datasets, we compared ML and BI reconstructions for the combined dataset (fig. 1.1) to a BI reconstruction for a dataset including ITS and PY-IGS for a greater number of taxa (fig. 1.2). Topology was relatively consistent at the genus level between reconstructions, with the exception of the position of *Drosophyllum*, which forms a polytomy with *Dioncophyllum* and *Triphyophyllum* with relatively high support (0.94 pp). It is also evident that the ITS and PY-IGS reconstruction does not fully resolve evolutionary relationships between taxa. This is especially true in Nepenthaceae, where ITS and PY-IGS are not phylogenetically informative enough to resolve relationships at the species level.

Ancestral reconstruction

Stochastic character mapping reveals the presence of non-vascularized, sessile glands alone (stalked and pitted glands absent) as the ancestral state for the carnivorous Caryophyllales (fig. 1.3A). Sessile glands were then lost in the lineage leading to Nepenthaceae and the clade containing Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae (0.94 pp). Within this group, vascularized, sessile glands containing both xylem and phloem arose secondarily and independently in *Drosophyllum* and *Triphyophyllum*. Within the taxa studied here, there are no known sessile glands associated only with xylem.

Stochastic character mapping also infers three independent origins of stalked glands within the ingroup (fig. 1.3B). Independently, the presence of stalked glands is an ancestral character state for outgroups (0.55 pp) and for Droseraceae (0.98 pp). The stalked glands of Droseraceae are vascularized with xylem, and according to our analyses these glands are secondarily lost in the flytrap lineages *Dionaea* and *Aldrovanda* (absence of stalked glands: 0.60 pp *Dionaea* – *Aldrovanda*, whereas 0.01 pp *Drosera*). In *Drosophyllum* and *Triphyophyllum*, stalked glands vascularized with xylem and phloem are gained independently.

According to our reconstruction results, pitted glands are gained independently by ingroup and outgroup taxa (fig. 1.3C). Pitted glands are found in *Limonium* and *Polygonum* and are reconstructed as the ancestral condition of the outgroup. Pitted glands are also found in Nepenthaceae and Ancistrocladaceae, and their presence is reconstructed as an ancestral character state (0.58 pp) for the clade containing Nepenthaceae and members of Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae. However, in this case, pitted glands are secondarily lost in Drosophyllaceae (currently absent in *Drosophyllum*) and the Dioncophyllaceae (0.90 pp). Pitted glands are non-vascularized in all taxa sampled in our analyses.

1.4 Discussion

Assessing phylogenetic relationships among the carnivorous Caryophyllales

Bayesian and maximum likelihood inference of phylogeny of the Caryophyllales and closely-related taxa based on the combined dataset revealed a tree topology similar to previous molecular studies, which includes a single gene analysis of *matK* (Meimberg et al. 2000) and combined analyses of 18S, *rbcL*, *atpB*, and *matK* (Cameron et al. 2002).

The relationship presented here of a monophyletic *Drosera* with *D. regia* as the closest living ancestor to the remainder of sampled *Drosera* species is incongruent with previous *matK* and *rbcL* single gene phylogenies, yet is consistent with 18S, *rbcL*, *atpB*, and *matK* topologies presented in the same study (Cameron et al. 2002). Our analyses are also inconsistent with a *rbcL* study of primarily *Drosera* (Rivadavia et al. 2003), where *Drosera* was found to be polyphyletic due to *D. regia* and *Aldrovanda* forming a clade sister to the remaining *Drosera* species, with *Dionaea* sister to all remaining Droseraceae. A multiple gene study (Cameron et al. 2002) is the most similar to our topology with regards to Droseraceae relationships, however our eight molecular marker analysis provides higher support for the monophyly of *Drosera* (88 bs/1.00 pp), as well as the sister relationship between *Dionaea* and *Aldrovanda* (100 bs/1.00 pp). Our analyses are also inconsistent with a *rbcL* study of primarily *Drosera* (Rivadavia et al. 2003), where *Drosera* was found to be polyphyletic due to *D. regia* and *Aldrovanda* forming a sister relationship just outside the entirety of sampled *Drosera*, and *Dionaea* was the closest living relative to all remaining Droseraceae.

Single gene analyses of *rbcL* (Fay et al. 1997; Lledó et al. 1998) both included sufficient taxa to test generic relationships across the carnivorous Caryophyllales. In Fay et al. (1997), Nepenthaceae was reconstructed as sister to a clade containing Droseraceae and members of the families Plumbaginaceae and Polygonaceae. The remaining families (Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae), were recovered as sister to this clade. In Lledó et al. (1998), Nepenthaceae was recovered as sister to Droseraceae and Droseraceae sister to a clade containing Drosophyllaceae, Dioncophyllaceae, and Ancistrocladaceae. Both analyses are inconsistent with the tree topology of our eight molecular marker analyses (fig. 1.1).

There has been uncertainty in the phylogenetic placement of Nepenthaceae in almost all past analyses of the Caryophyllales (Cameron et al. 2002; Cuénoud et al. 2002; Fay et al. 1997; Nandi et al. 1998; Soltis et al. 2000). The exception is a single gene analysis of *matK* (Meimberg et al. 2000), in which Nepenthaceae and a clade containing Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae is sister to Droseraceae with moderate support. In our eight molecular marker analyses, Nepenthaceae as sister to the clade containing Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae is highly supported (89 bs/1.00 pp). In addition, analyses in the past show little support for the reconstruction of Nepenthaceae and Droseraceae as sister clades (Cuénoud et al. 2002; Fay et al. 1997; Hilu et al. 2003; Lledó et al. 1998; Nandi et al. 1998; Soltis et al. 2000). In contrast, combined and ITS and PY-IGS analyses show strong support for Droseraceae as sister to the clade containing Nepenthaceae, Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae (100 bs/1.00 pp).

Drosophyllum was previously thought to be allied with the Droseraceae (Cronquist 1988), an idea supported by an early *rbcL* analysis (Williams et al. 1994) that placed *Drosera* sister to *Drosophyllum*. A later analysis of *rbcL* (Lledó et al. 1998) also suggested a relationship between *Drosophyllum* and Droseraceae; one of their three equally most parsimonious trees depicted Droseraceae as sister to a clade that included *Drosophyllum*, *Ancistrocladus* and *Triphyphyllum*.

Since this time, *Drosophyllum* has been separated from Droseraceae and moved into the monotypic Drosophyllaceae based upon several multiple-locus phylogenies that suggest a sister relationship between *Drosophyllum* and the Nepenthaceae (APGII 2003). Our analyses clearly separate *Drosophyllum* from both Droseraceae and Nepenthaceae, placing it in a moderately well-supported clade with *Ancistrocladus*, *Dioncophyllum*, and *Triphyophyllum* (fig. 1.1). In our ITS and PY-IGS BI phylogenetic reconstruction (fig. 1.2), *Drosophyllum* forms a polytomy with *Dioncophyllum* and *Triphyophyllum*. The placement in the ITS and PY-IGS analysis is likely an artefact of the smaller dataset not providing sufficient phylogenetically informative characters to resolve the relationship between *Dioncophyllum*, *Drosophyllum* and *Triphyophyllum*.

The relationship of *Ancistrocladus* as sister to *Dioncophyllum* and *Triphyophyllum* has very high support (100 bs/1.00 pp), similar to previous analyses that recover this relationship (Cameron et al. 2002; Cuénoud et al. 2002; Fay et al. 1997; Heubl et al. 2006; Hilu et al. 2003; Meimberg et al. 2000; Soltis et al. 2000). Meimberg et al. (2000) and Heubl et al. (2006) also recover *Dioncophyllum* as sister to *Triphyophyllum*, comparable to our results (100 bs/1.00 pp).

Topological incongruence observed among ours and previous phylogenetic reconstructions may be due to a sampling limitation of species per genus in previous studies and/or the amount of missing data in many of the combined molecular marker analyses (Wiens 2003). We tested for familial relationships through larger sampling of species within each genus, limiting the amount of missing molecular data, and using Bayesian and likelihood-based methods for phylogenetic reconstruction to decrease the potential for long-branch attraction (Bergsten 2005) especially considering the seemingly long evolutionary distances in some of the monotypic lineages (*Drosophyllum*, *Dionaea*, *Aldrovanda*) (figs. 1.1 and 1.2). As both our BI and ML analyses (ML branch lengths not shown in fig. 1.1) agree with the long branch associated with *A. vesiculosa*, it is unlikely that BI's sometimes inaccurate branch-length estimates are the cause (Brown et al. 2010). It is more likely that the long branch is a relic of extinction (Magallón 2010), as *A. vesiculosa* represents a larger lineage with a rich fossil record dating back to the early Tertiary (Degreef 1997).

Ancestral reconstruction of carnivorous glands of the Caryophyllales

The results of our phylogenetic reconstructions provide a backbone to investigate the evolution of the carnivorous habit at the level of the gland – a morphological feature of the carnivorous plant trap that allow for the secretion of enzymes and absorption of digested products (Amagase 1972, Amagase et al. 1972; Dexheimer 1978; Henry and Steer 1985; Stoltzfus et al. 2002). The presence of glands is a synapomorphy for the non-core Caryophyllales (Judd et al. 2002). Glands can either be sessile, stalked, or pitted, and while sometimes vascularized with xylem and phloem, the presence of vasculature within a gland is not an indicator of its functionality in carnivory.

Stochastic mapping of gland morphology resulted in sessile gland type as most likely ancestral when considering gland type alone, whether absent, sessile, stalked, or pitted (fig. 1.3A). Sessile glands containing xylem and phloem evolved independently in *Drosophyllum* and *Triphyophyllum*, a feature absent from all other sessile glands found in Droseraceae, Plumbaginaceae, and Polygonaceae (fig. 1.3A). Stalked and pitted glands are gained independently by ingroup and outgroup taxa, with lower posterior probability values for the occurrence of these glands as ancestral character states for *Limonium* and *Polygonum*. These results could be due to the lack of knowledge and inclusion of characters related to gland functionality in our analyses.

Results from stochastic character mapping imply that the evolution of stalked glands with

xylem-containing vasculature (Droseraceae) and those that contain both xylem and phloem (*Drosophyllum* and *Triphyophyllum*) occurred as separate events (fig. 1.3B). In addition, according to these results it is also highly unlikely (0.12 pp) that the evolution of glands containing both xylem and phloem occurred in the common ancestor to the clade comprising Ancistrocladaceae, Dioncophyllaceae, and Drosophyllaceae. Instead, the evolution of glands with both xylem and phloem was likely to have occurred twice, once in the lineage leading to the extant full-time carnivore *Drosophyllum* and a second time in the lineage leading to part-time carnivore *Triphyophyllum* after the divergence of the *Dioncophyllum* lineage. Additional studies investigating the development and secretion chemistry of stalked glands in *Drosophyllum* and *Triphyophyllum* would help shed light on this apparent homoplasy.

Stochastic character mapping also demonstrates the loss of stalked glands by the common ancestor of the flytraps *Dionaea* and *Aldrovanda* (fig. 1.3B). These taxa are considered carnivorous and retain sessile glands without associated vasculature. It has been proposed that vascularized, stalked multicellular glands may have been reduced to teeth and trigger hairs during the evolution of the lamina—a hypothesis with supporting evidence derived by S.E. Williams (1976) and revisited by T.C. Gibson and D.M. Waller (2009). This hypothesis is consistent with our reconstruction of ancestral gland character states, where stalked glands have been lost in *Dionaea*, while teeth and trigger hairs have been gained as a morphological character (data not coded). Like in *Dionaea*, the teeth and trigger hairs of the *Aldrovanda* trap may be derived from vascularized, stalked glands resulting in the apparent loss of these glands. If so, this conversion of vascularized, stalked glands to trigger hairs and teeth could have occurred in the common ancestor of *Dionaea* and *Aldrovanda*.

It is evident that the evolution of the vascularized gland is a novel feature of the carnivorous Caryophyllales, as only *Drosera*, *Drosophyllum*, and *Triphyophyllum* have vascularized glands involved in carnivory. In some genera of the Caryophyllales we found a lack of association between gland vasculature and carnivory (i.e. *Dionaea*, *Nepenthes*), as not all carnivorous plants have vascularized glands. It is also apparent that sessile and stalked glands are not required for carnivory in *Nepenthes*. Our investigations of gland morphology among carnivorous taxa and closely related non-carnivorous taxa therefore demonstrate that the internal architecture of glands on the lamina is not indicative of plant carnivory.

On the origin of carnivorous glands in the Caryophyllales

It is clear that plant carnivory is independent of the presence of vascularized glands in *Dionaea*, *Aldrovanda*, and *Nepenthes* (fig. 1.3A-C), as the absorption of nutrients by non-vascularized glands has been exemplified within *Dionaea*, *Aldrovanda*, and *Nepenthes* (An et al. 2002; Fabian-Galan and Salageanu 1968; Robins and Juniper 1980). This prompts two questions: how and why did vascularized glands arise in *Drosera*, *Drosophyllum*, and *Triphyophyllum*?

Vascularized, stalked multicellular glands that excrete enzymes and absorb nutrients for plant carnivory could have evolved by a number of methods, two of which could have been either by pinnation or emargination of the leaf blade (fig. 1.4). If by pinnation, marginal glands were formed by homologous transformation of pinnae into vascularized, stalked glands. Alternatively, emargination of the leaf blade could have occurred, whereby marginal glands evolved through gland definition. After the formation and evolution of vascularized, stalked multicellular glands, the ancestors of extant carnivorous Caryophyllales diversified and radiated through modification or reduction of stalked glands (Gibson and Waller 2009; Juniper et al. 1989). During three separate

events, stalked glands with vasculature were acquired by ancestors of Droseraceae, Drosophyllaceae, and Dioncophyllaceae, leading to extant carnivorous taxa in the genera *Drosera*, *Drosophyllum*, and *Triphyophyllum*. Reduction of vascularized, stalked glands is hypothesized to have occurred in ancestors of extant *Aldrovanda* and *Dionaea*, whereby vascularized, stalked glands were reduced to vascularized teeth at the margin of the trap, as well as trigger hairs on the abaxial side of the leaf surface (Gibson and Waller 2009; Williams 1976). Stochastic character mapping for pitted, sessile, and stalked glands supports the loss of stalked glands at the ancestral node to *Dionaea* and *Aldrovanda* (fig. 1.3B).

Evidence to support pinnation, or the homologous transformation of pinnae to marginal glands, comes from studies of *Drosera* leaf development. In *D. capensis* seedlings, marginal glands that resemble pinnae can be found on cotyledons (Diels 1906). Interestingly, some closely related living members to the carnivorous Caryophyllales exhibit pinnately lobed leaves (e.g. Plumbaginaceae, *Limonium imbricatum*). If this type of leaf morphology is present in the Caryophyllales, it is possible that an ancestor to the carnivorous plants could have evolved pinnately compound leaves and secondarily evolved marginal glands through rearrangement of leaf architecture. Alternatively, emarginate leaves are found in some Polygonaceae (*Rheum*), a sister family to Plumbaginaceae, and the study of this leaf type may provide clues as to another way by which marginal glands evolved.

A greater understanding of the evolutionary origin of carnivorous glands and structures involved in plant carnivory could stem from developmental genetic studies. For example, assessing which genes are expressed within the gland during its development may help to elucidate vascularized, stalked gland homology. Homologies in leaf form inferred from gene expression have been demonstrated in both angiosperms and gymnosperms (Bharathan et al. 2002; Eckardt 2007; Floyd and Bowman 2009). Similarly, expression profiling of carnivorous glands from the non-core Caryophyllales could allow us to escape misinterpretations of homologies within leaf morphology.

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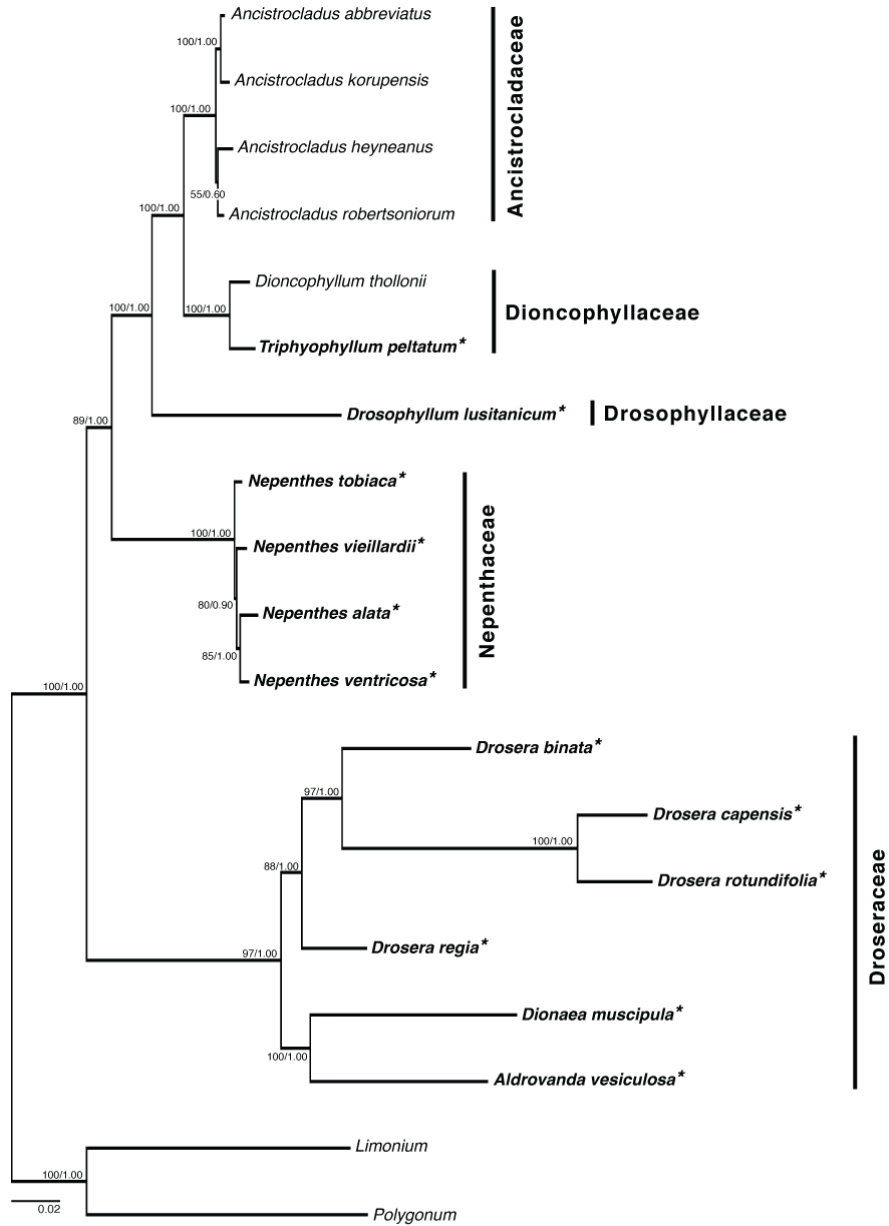


Fig. 1.1 Combined nDNA, nrDNA, and cpDNA molecular marker phylogenetic reconstruction for the carnivorous Caryophyllales. Results from ML and BI analyses of a concatenated ITS, PY-IGS, *atpB*, *petB*, *matK*, PTR1, *rbcL*, *trnK* dataset for 19 taxa. Posterior probabilities and bootstrap support from complete analysis are indicated (BS/PP) at nodes on the Bayesian 50% majority rule tree. Carnivorous taxa are indicated by an asterisk and are in bold typeface.

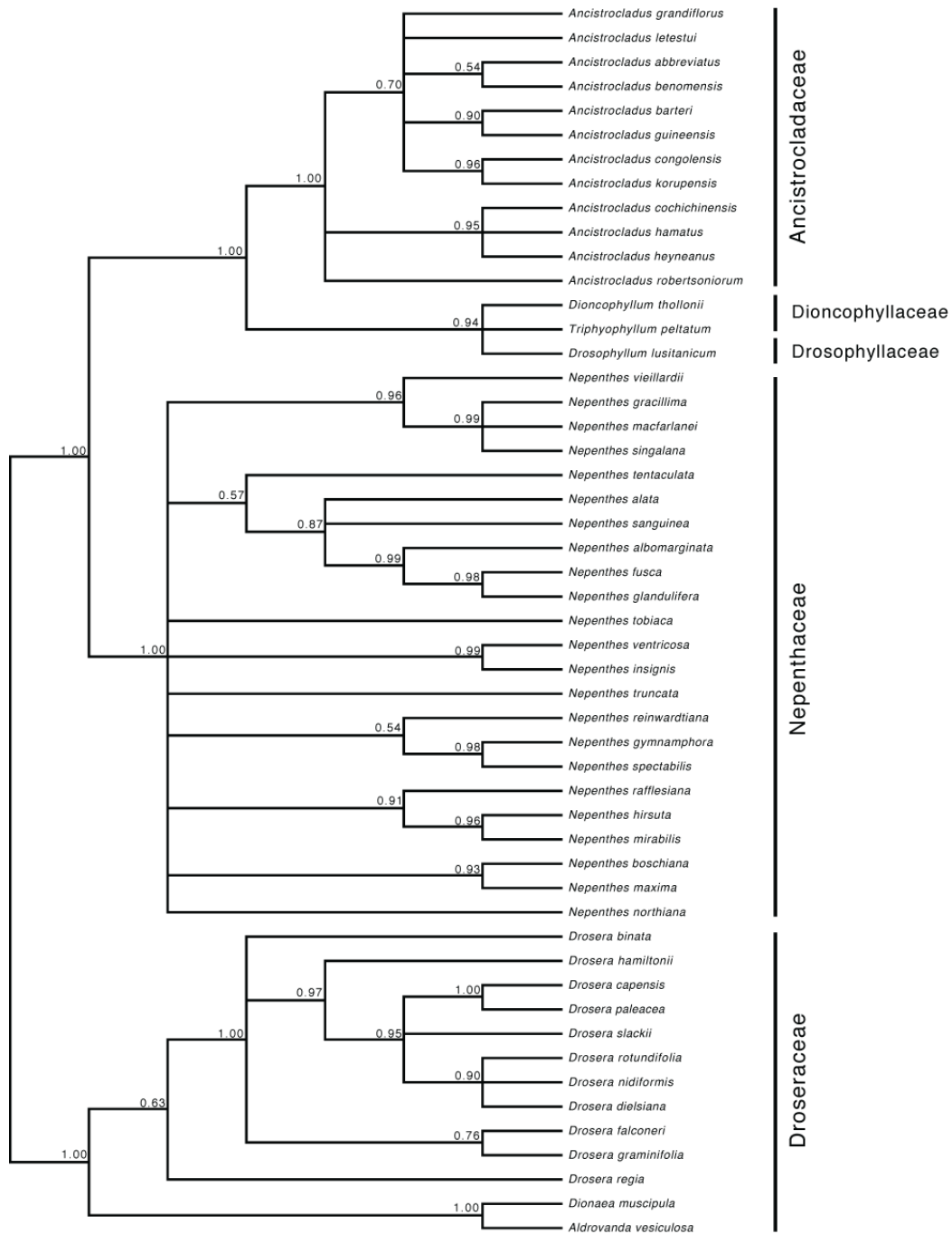


Fig. 1.2 ITS and PY-IGS phylogenetic reconstruction for the carnivorous Caryophyllales. Results from BI analyses of ITS and PY-IGS for 51 taxa. Posterior probabilities from complete analysis are indicated at nodes on the Bayesian 50% majority rule tree.

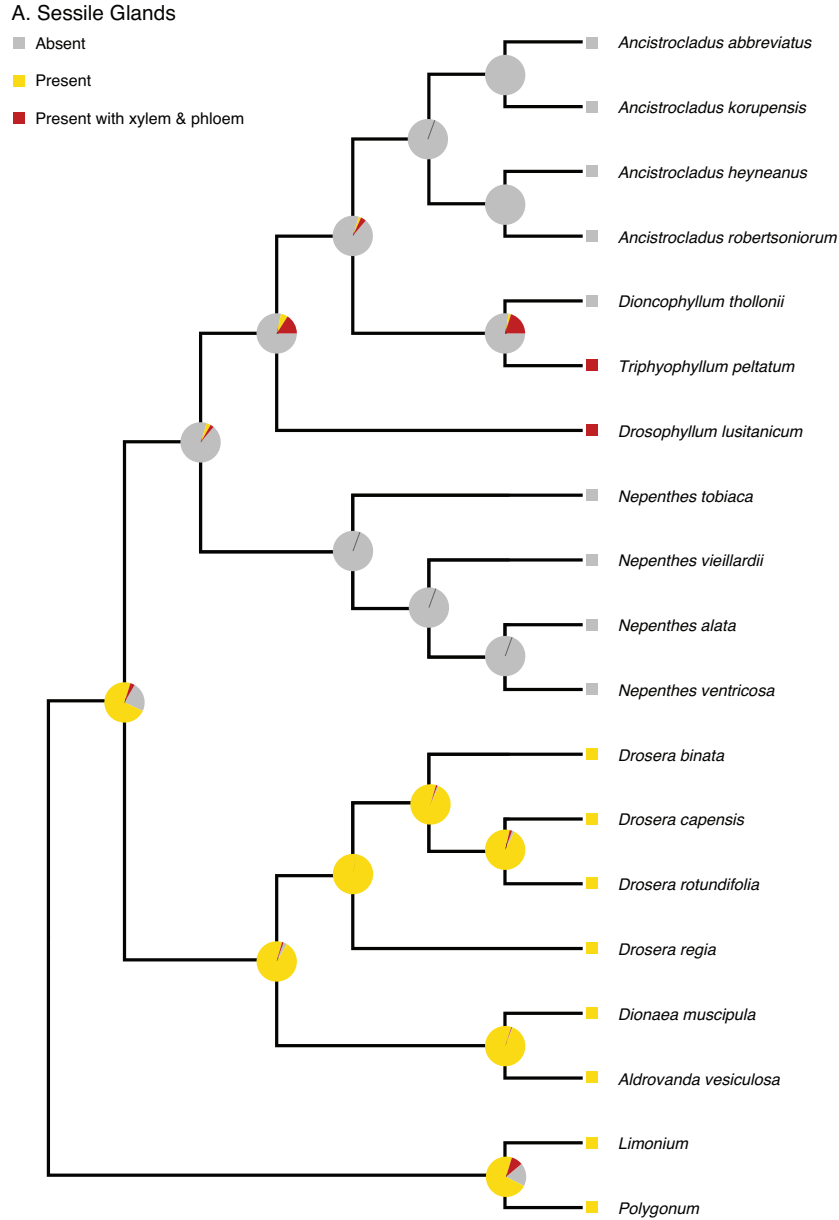


Fig. 1.3 A-C Stochastic character mapping of gland states associated with plant carnivory. Stochastic character mapping of gland types and associated vasculature in SIMMAP utilizing the 50% majority rule tree assembled from BI phylogenetic reconstruction of the concatenated ITS, PHYGS, *atpB*, *matK*, *petB*, PTR1, *rbcL*, *trnK* dataset for 19 taxa. Pie charts at nodes represent ancestral states at each node that were calculated as the marginal posterior probability of each possible character state. Three basic gland types and associated vasculature found in carnivorous Caryophyllales and closely related non-carnivorous taxa were mapped: (A) sessile glands, (B) stalked glands, (C) pitted glands.

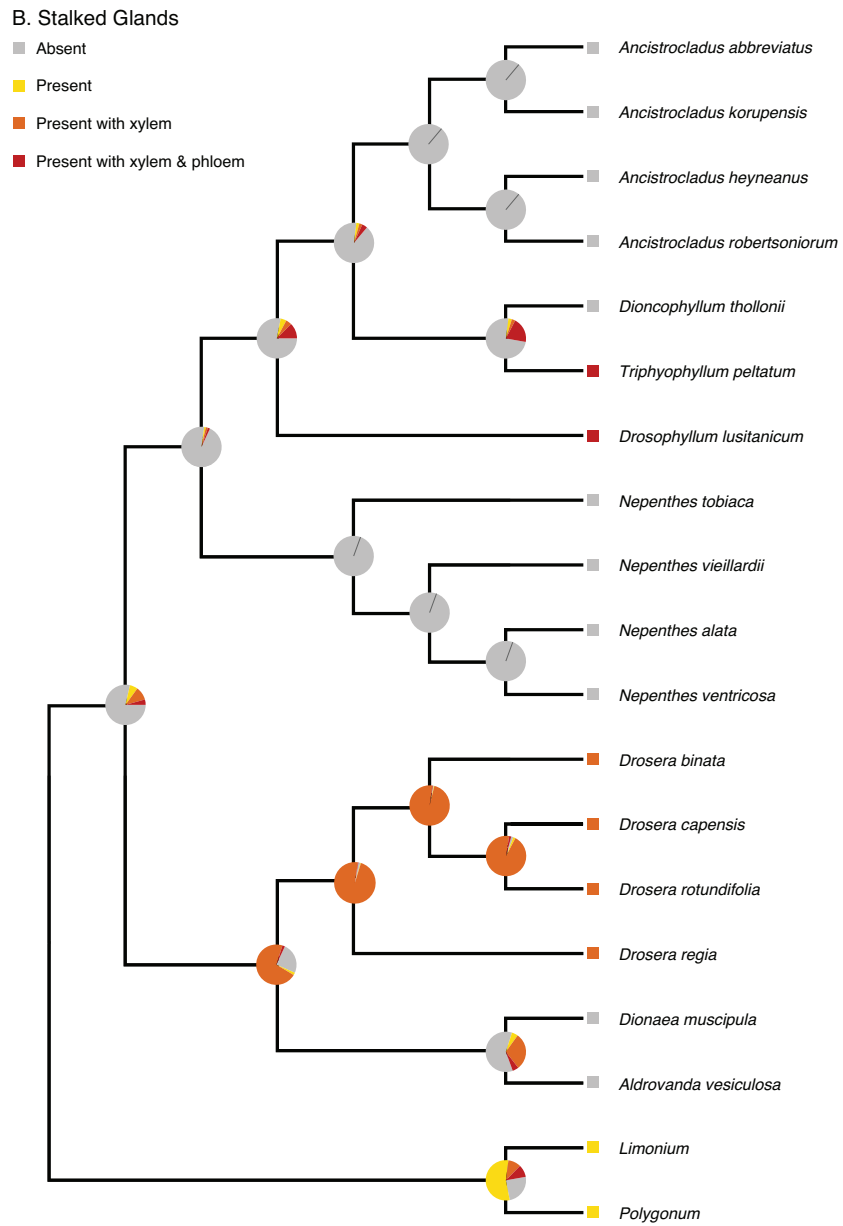


Fig. 1.3 continued.

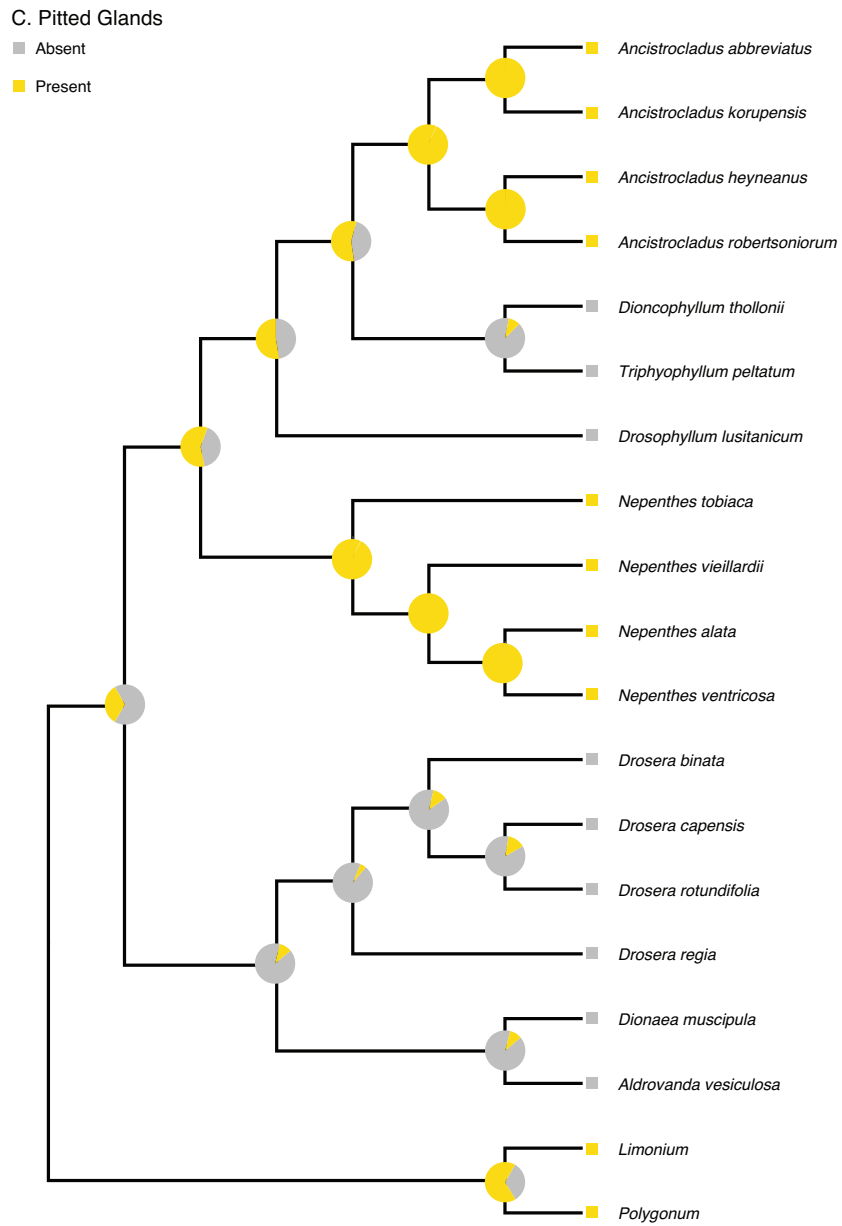


Fig. 1.3 continued.

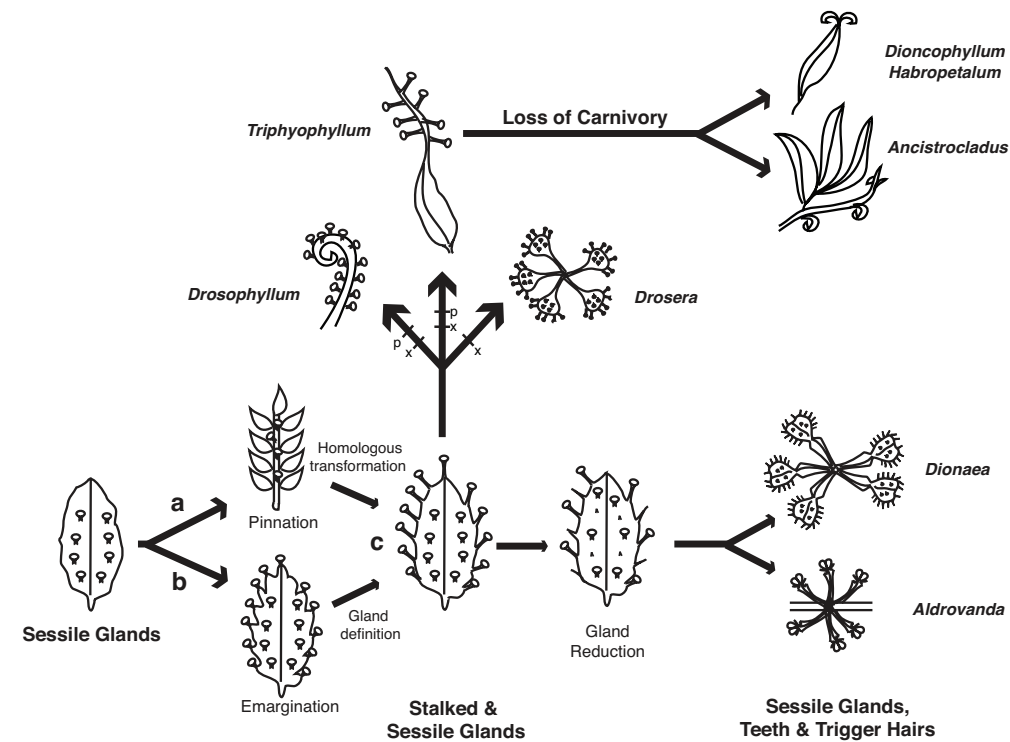


Fig. 1.4 Pinnation and emargination pathways for the evolution of the carnivorous gland in the Caryophyllales. Shown are two alternative pathways for the evolution of the carnivorous gland: following pinnation, marginal glands were formed by homologous transformation of pinna into vascularized stalked glands (*a*); as an alternative, emargination of the leaf blade occurred, and marginal glands evolved at the leaf margins (*b*). After the formation and evolution of stalked glands, the Caryophyllales diversified and radiated into the carnivorous taxa known today through retainment or reduction of glands (*c*). Extant taxa have sessile, stalked, or pitted glands. Teeth and trigger hairs, two unique morphological features of *Dionaea* and *Aldrovanda*, respectively, may be homologous to stalked glands. The three separate vascularization events in ancestors of *Drosera*, *Drosophyllum*, and *Triphyphyllum* are indicated, and the presence of xylem and/or phloem is represented by *x* (xylem) and *p* (phloem).

Chapter Two

Molecular and functional evolution of class I chitinases for plant carnivory in the Caryophyllales

Intended for future submission as a research article:

Renner T, Specht CD. Molecular and functional evolution of class I chitinases for plant carnivory in the Caryophyllales.

Abstract

Proteins produced by the large and diverse chitinase gene family are involved in the hydrolyzation of glycosidic bonds in chitin, a polymer of *N*-acetylglucosamines. In flowering plants, class I chitinases are important pathogenesis-related (PR) proteins, functioning in the determent of herbivory and pathogen attack by acting on insect exoskeletons and fungal cell walls. Within carnivorous plants, two subclasses of class I chitinases have been identified to play a role in the digestion of prey. Members of these two subclasses, depending on the presence or absence of a C-terminal extension, can be secreted from specialized digestive glands found within the morphologically diverse traps that develop from carnivorous plant leaves. The degree of homology among carnivorous plant class I chitinases and the method by which these enzymes have been adapted for the carnivorous habit has yet to be elucidated. This study focuses on understanding the evolution of carnivory and chitinase genes in one of the major groups of plants that has evolved the carnivorous habit: the Caryophyllales. We recover novel class I chitinase homologs from species of genera *Ancistrocladus*, *Dionaea*, *Drosera*, *Nepenthes*, and *Triphyophyllum*, while also confirming the presence of two subclasses of class I chitinases based upon sequence homology and phylogenetic affinity to class I chitinases available from sequenced angiosperm genomes. We further detect residues under positive selection and reveal substitutions specific to carnivorous plant class I chitinases. These substitutions may confer functional differences as indicated by protein structure homology-modeling.

2.1 Introduction

Plants have evolved a wide array of methods to protect themselves against pathogens, including the production of pathogenesis-related (PR) proteins. Chitinases, which comprise four of the PR families (PR-3, PR-4, PR-8, and PR-11) are either induced in direct response to insect herbivory and fungal elicitors or are constitutively expressed in tissues vulnerable to attack (Brogue et al. 1988; Samac et al. 1990). The chitinase catalytic mechanism involves the hydrolysis of β -1,4-glycosidic linkages between *N*-acetylglucosamine (NAG) oligomers in chitin polymers, the major constituents of fungal walls and arthropod exoskeletons. Chitinases are further divided into families 18 and 19 of glycoside hydrolases (GH) based on amino acid sequence similarity (Li and Greene 2010). Members of the two chitinase families have been identified in all plants analyzed to date, many of which have

been shown to inhibit fungal growth *in vitro* (Schlumbaum et al. 1986; Leah 1991) and enhance resistance to fungal pathogens in transgenic plants (Brogue et al. 1988; Jach 1995; Eilenberg et al. 2006).

Although functionally related, members of these two chitinase families do not share sequence similarity or three-dimensional structure and the two families are hypothesized to have arisen independently (Hamel et al. 1997). GH families 18 and 19 are encoded by large gene families and have been organized into five classes according to sequence, structure, and phylogenetic relationship (Shinshi et al. 1990; Collinge et al. 1993; Beintema 1994; Melchers et al. 1994; Araki and Torikata 1995; Neuhaus 1996; Hamel et al. 1997; Passarinho and de Vries 2002): classes I, II, and IV comprise family 19, and classes III and V together form GH family 18.

GH family 19 chitinases, which are able to bind to and catalyze the hydrolysis of chitin polymers, are responsible for the majority of chitinolytic activity within plant material (Legrand et al. 1987). These include the class I chitinases, characterized by four consecutive domains: (1) a hydrophobic but variable N-terminal signal peptide, (2) a cysteine-rich domain, (3) a highly variable proline-rich hinge, and (4) a catalytic domain (Iseli et al. 1993; Melchers et al. 1993; Graham and Sticklen 1994). Processing of class I chitinase preproteins occurs at the endoplasmic reticulum, where the N-terminal signal peptide is removed from the mature form. The cysteine-rich domain is involved in chitin-binding and certain positions within the chitin recognition or binding domain signature PS00026 (C-x(4,5)-C-C-S-x(2)-G-x-c-g-x(4)-[FYW]-C) are highly conserved. Separating the cysteine-rich domain from the catalytic domain is the highly variable proline-rich hinge, a region that not only varies in sequence but length, and may be absent altogether. Within the catalytic domain, positions are conserved between class I chitinases within the chitinase 19_1 signature PS00773 (C-x(4,5)-F-Y-[ST]-x(3)-[FY]-[LIVMF]-x-A-x(3)-[YF]-x(2)-F-[GSA]) and chitinase 19_2 signature PS00774 ([LIVM]-[GSA]-F-x-[STAG](2)-[LIVMFY]-W-[FY]-W-[LIVM]). In addition to these domains, a C-terminal extension (CTE) may also be present, a sequence that has been shown to be necessary and sufficient for transmission of the protein to the vacuole (Neuhaus et al. 1991; Chrispeels and Raikhel 1992). When the CTE is absent or is altered via mutagenesis, class I chitinases are translocated extracellularly (Esaka et al. 1990; Dore et al. 1991; Melchers et al. 1993; Neuhaus et al. 1994). The presence or absence of a CTE thus divides class I chitinases into two subclasses: subclass Ia (CTE present) and subclass Ib (CTE absent).

Class I chitinases play a major role in pathogenic response in plants (Wu et al. 1994; Gijzen et al. 2001; Zhao et al. 2011) and evidence for the rapid adaptive evolution of these genes exists for both eudicot and monocot class I chitinases (Bishop et al. 2000; Tiffen 2004). In *Arabidopsis*, adaptive amino acid replacements occur disproportionately in the active site cleft (Bishop et al. 2000), while positively selected amino acids have been identified within a homologous active site cleft in *Zea* class I chitinases (Tiffen 2004). The presence of positively selected sites has been attributed to an evolutionary arms race between class I chitinases and competitive inhibitors produced by fungal pathogens. Differences in the number of positively selected sites for certain flowering plant lineages may reflect lineage-specific adaptive responses.

In addition to serving as a pathogenic response in plants, class I chitinases have been shown to be important players in plant carnivory. Chitinase enzyme activity in the presence of chitin was demonstrated in the Caryophyllales within the specialized carnivorous traps of sundews (*Drosera*) and tropical pitcher plants (*Nepenthes*). To validate that chitinolytic activity was endogenous, plant cDNA, genomic, and protein sequences were obtained and expression was localized to the digestive glands (Matusíková et al. 2005; Eilenberg et al. 2006). In *Nepenthes khasiana*, subclass Ia and Ib chitinases were shown to be differentially expressed in the secretory region of the trap. Subclass Ia is

constitutively expressed in the secretory cells, whereas subclass Ib expression was upregulated in response to chitin (Eilenberg et al. 2006). Sequence dissimilarity between these subclasses is attributed to the presence of a variable proline-rich hinge and loss of a vacuole targeting signal at the carboxyl terminal in subclass Ib, the latter hypothesized to allow excretion from the secretory cells for prey digestion (Eilenberg et al. 2006). In *Drosera rotundifolia*, class I chitinases were localized to the sessile and stalked glandular tentacles utilized in prey trapping and digestion and, similar to subclass Ib chitinases in *Nepenthes*, were shown to be upregulated in response to chitin substrate. Class I chitinases thus seem to play a double role in carnivorous plants, both for pathogenic response and prey digestion.

While the evolution of class I chitinases has been studied in non-carnivorous plants in relation to pathogenic-response (Bishop et al. 2000; Tiffen 2004), this study is the first to analyze the relationship between the evolution of the chitinase gene family and plant carnivory. We identify homologs of subclass Ia and Ib chitinases in the Caryophyllales from *Drosera*, *Dionaea*, a closely related part-time carnivorous plant (*Triphyophyllum*) and related plants that have lost the carnivorous habit (*Ancistrocladus*) (Renner and Specht 2011). In addition, we test whether subclasses of class I chitinases in the carnivorous Caryophyllales have a similar proportion of residue replacements in the active site cleft as class I chitinases primarily utilized as PR proteins by non-carnivorous monocots and eudicots. If a certain subclass of chitinase is primarily used for prey digestion rather than for pathogenic response, selection may act differently on these proteins. Our research is driven by two hypotheses: (1) during the evolution of carnivory within the Caryophyllales, chitinase genes that were primarily utilized for pathogenesis response by ancestral non-carnivorous plants diverged, allowing for subfunctionalization of subclass Ia (for pathogenesis) and Ib chitinases (for carnivory), and (2) selection pressure acting on subclass Ib chitinases shifted as functional pathogenesis response gave way to a role specific to carnivory.

2.2 Materials and Methods

Tissue collection and DNA extraction

Class I chitinases were amplified from species within the families Ancistrocladaceae, Dioncophyllaceae, Droseraceae, and Nepenthaceae (appendix). Collected tissue samples originated mainly from living collections at California Carnivores in Sebastopol, California, Missouri Botanical Garden, University of California Botanical Garden, and Universität Würzburg. Freshly collected leaf tissue was preserved in silica gel and frozen at -80°C . Total genomic DNA was extracted from the leaf base (*Dionaea*), leaf lamina (*Ancistrocladus* and *Drosera*) and the lamina-like region of the leaf-base (*Nepenthes* and *Triphyophyllum*) using cetyltrimethyl ammonium bromide (CTAB) (Doyle and Doyle 1987) or a modified sodium dodecyl sulfate (SDS) and sodium chloride protocol (Edwards et al. 1991). Extracted genomic DNA was quantified with a NanoDrop 1000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Genomic DNA was chosen over cDNA for the ability to use intergenic regions to distinguish multiple gene copies from allelic variability, the capability to isolate pseudogenes, and the potential that certain subclasses were not be expressed in all tissue obtained for cDNA synthesis. Taxa utilized in our analyses are summarized in A.4.

PCR and cloning of class I chitinase genes

Four sets of primer pairs were designed from full-length *Nepenthes khasiana* gDNA sequences (AY618881, AY618883, AY618885, AY618887), and partial subclass Ib mRNA sequences from Droseraceae (AY643484, AY622818, AY643483) available in GenBank. Subclass Ia chitinase genes were amplified using primer pairs PFF 509 (5'-CCA-AAC-TTC-CCA-TGA-AAC-TAC-3')/PFF 511 (5'- CCC-CAC-CGT-TGA-TGA-TGT-T-3') and PFF 757 (5'-GCC-AGA-GCC-AGT-GCA-GC-3')/PFF 758 (5'- CCC-ACC-GTT-GAT-GAT-GTT-3'). Subclass Ib chitinase genes were amplified with primer pairs PFF 755 (5'- TGC-CAG-AGC-CAG-TGT-GG-3')/PFF 756 (5'- CTG-GMT-GTA-GCA-GTC-CAA-GTT-3'). PCRs were performed in 10 µl aliquots with 10-100 ng of genomic DNA, 0.02 U iProof™ Polymerase (Bio-Rad, Hercules, CA, USA), 1X HF iProof buffer, 2.0 mM MgCl₂, 0.2 mM each of dNTP, 0.5 µM of each primer and 100% DMSO. When the iProof™ Polymerase system failed to amplify PCR products using the designed primer pairs, the Phire® Plant Direct PCR Kit (Finnzymes Inc., Woburn, MA, USA) was used. All PCR reactions were run on a MyCycler (Bio-Rad) thermal cycler under a three-step PCR protocol as suggested by the manufacturer.

Amplified products were either directly cloned or gel-purified with a QIAquick gel extraction kit (QIAGEN, Germantown, MD, USA) and subsequently cloned into the pJet1.2 vector (Fermentas International Inc., Burlington, Ontario, Canada). At least 8 colonies per ligation reaction were chosen to screen with colony PCR. Sequences from the resulting colonies were amplified with vector-specific primers and the Phire® Polymerase (Finnzymes Inc., Woburn, MA, USA). PCR products were purified using exonuclease I and shrimp alkaline phosphatase to remove single-stranded primers and remaining dNTPs (Fermentas International Inc., Burlington, Ontario, Canada) and cycle sequenced using described PCR primers (2 µM) and the ABI Prism BigDye Terminator Cycle Sequence Ready Reaction Kit v3.1 (Perkin-Elmer/Applied Biosystems, Foster City, CA, USA). Products of cycle sequencing were resolved on an ABI Prism 3100 or 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). Caryophyllales subclass Ia and Ib chitinase gDNA sequences (table 2.1) are available via GenBank (Benson et al. 2005), with the exception of sequences >200bp, which are available in A.5.

The percentage of identical sites between translated *N. khasiana* complete subclass Ia and Ib chitinases (AY618881, AY618883, AY618885, AY618887) and partial class I chitinases amplified from Nepenthaceae, Droseraceae, Ancistrocladaceae, and Dioncophyllaceae, was calculated in Geneious Pro 5.3.6 (Drummond et al. 2010; available at <http://www.geneious.com/>). Perfect and imperfect tandem repeats were located in Caryophyllales subclass Ia and Ib chitinase sequences using Phobos 3.3.12 (Mayer 2006-2010).

Identification of class I chitinase homologs

Two bioinformatic approaches were used in combination to identify homologs of carnivorous plant class I chitinases from online databases. First, *Nepenthes khasiana* subclass Ia and Ib chitinases (Genbank AY618881, AY618883, AY618885, AY618887) were used as query sequences for CoGeBlast (Lyons and M. 2008; Lyons et al. 2008) tblastx searches against the following genomes: *Arabidopsis thaliana* (TAIR: v9, masked repeats 50x), *Brachypodium distachyon* line Bd21 (JGI: v1, masked repeats 50x), *Glycine max* (JGI: v1, masked repeats 50x), *Lotus japonicus* (v1, unmasked), *Medicago truncatula* (Medicago.org: v3.5.1, unmasked), *Oryza sativa* ssp. *japonica* (MSU Rice

Genome Annotation: v6.1 masked repeats 50x), *Physcomitrella patens* (JGI: v1.1, unmasked), *Populus trichocarpa* (JGI: v2 masked by JGI v1.4), *Selaginella moellendorffii* (JGI: v1, unmasked), *Sorghum bicolor* (JGI: v1.4, masked repeats 50x), *Vitis vinifera* (French National Sequence Center: v2 masked by genoscope), *Zea mays* ssp. *mays* (MaizeSequence.org: refgen_v2 assembly, filtered gene set annotations: 5b, v2 super masked repeats 50x). Only hits with an E-value cut-off of less than 0.001 and the most complete gene model for each chromosomal location were retained. An alignment of class I chitinases identified by CoGeBlast was constructed with ClustalX under default settings (Thompson et al. 1994), adjusted manually, and subsequently translated in Mesquite v2.72 (Maddison and Maddison 2010).

HMMER v3.0 (<http://hmmer.janelia.org/>) was then used to create the HMM profile 'krEiAAFLaQTSHETTgGWatAPdGpYaWGYCf' to surround the H-E-T-T motif, a signature highly conserved among class I chitinases (Passarinho and de Vries 2002) to search against annotated *A. thaliana*, *O. sativa* ssp. *japonica*, *S. bicolor*, and *V. vinifera* genomes. Corresponding gene hits obtained with HMMER from the specified plant genomes were downloaded from Phytozome v7.0 (<http://www.phytozome.net/>). Any class I chitinases found in addition to those retrieved from CoGe searches were included in our analyses.

As class IV chitinases can be remarkably similar in their sequence and domain structure to class I chitinases, a set of criteria were developed to ensure only class I chitinases were saved from CoGeBlast and HMMER searches. Sequences from genome searches were retained only if the following signatures were present: (1) chitinase-binding domain (Prosite PS00026), (2) Chitinase 19_1 signature (Prosite PS00773), (3) Chitinase 19_2 signature (Prosite PS00774), and (4) H-E-T-T motif.

Five class IV chitinases from eudicots and monocots (AtChitIV, AT2G43590; NaChitIV, GenBank AB289807; OsChitIV, LOC_Os02g39330; SbChitIV, Sb06g021220; VvChitIV, GSVIVG01038117001) serve as the outgroup for analyses of the HMM-derived dataset. Class IV chitinases have a similar domain architecture to class I, but are significantly shorter due to deletions within the chitin-binding and catalytic domains (see supplementary S1 and S2, Supplementary Material online). In addition, for each class IV sequence, the proline-rich hinge is absent and amino acid substitutions occur in the H-E-T-T motif of the first active site of the catalytic domain resulting in H-E-T-G/I. All identified class IV chitinases are missing a CTE and are therefore presumed to be extracellular.

Class I chitinase nomenclature

To easily refer to the proteins discovered via the methods described, we have designated the following naming system: for those class I chitinases from *Ancistrocladus grandiflorus*, AgChitI-x; *Ancistrocladus robertsoniorum*, ArChitI-x; *Arabidopsis thaliana*, AtChitI-x; *Brachypodium distachyon*, BdChitI-x; *Dionaea muscipula*, DmChitI-x; *Drosera binata*, DbChitI-x; *Drosera capensis*, DcChitI-x; *Drosera rotundifolia*, DrChitI-x; *Drosera spathulata*, DsChitI-x; *Glycine max*, GmChitI-x; *Lotus japonicus*, LjChitI-x; *Medicago truncatula*, MtChitI-x; *Nepenthes khasiana*, NkChitI-x; *Nepenthes maxima*, NmaxChitI-x; *Nepenthes mirabilis*, NmirChitI-x; *Oryza sativa* ssp. *japonica*, OsChitI-x; *Populus trichocarpa*, PtChitI-x; *Sorghum bicolor*, SbChitI-x; *Triphyophyllum peltatum*, TpChitI-x; *Vitis vinifera*, VvChitI-x; *Zea mays* ssp. *mays*, ZmChitI-x. The letter x denotes the number associated with the genomic DNA (gDNA) sequence (table 2.2).

Phylogenetic analysis

Forward and reverse sequences for newly amplified class I chitinase genes from the Caryophyllales were assembled and edited with Sequencher v4.7 (Gene Codes Corp.). A multiple sequence alignment for 49 chitinase sequences was constructed with ClustalX under default settings (Thompson et al. 1994) with manual adjustment in Mesquite v2.72 (Maddison and Maddison 2010). Regions that were poorly aligned across the entire dataset were removed from the CDS translation for phylogenetic analyses. These include intergenic regions, the proline-rich hinge between chitin-binding and catalytic domains, and N- and C- termini. For subsequent analyses, we used two datasets to assess class I chitinase evolution: (1) a protein alignment including all class I chitinase homologs identified in angiosperm genomes studied here and those retrieved from the Caryophyllales either via PCR or through GenBank (“angiosperm-wide”), and (2) a reduced dataset only including HMM-derived *A. thaliana*, *V. vinifera*, *O. sativa* ssp. *japonica*, and *S. bicolor* class I chitinase homologs and those obtained from the Caryophyllales (“HMM-derived”). Class I chitinase sites and corresponding residues described here are numbered according to positions within these multiple sequence alignments (A.5). The seven active sites (I-VII) of the catalytic domain are also indicated in A.5, which correspond to regions described by Garcia-Casado et al. (1998), Bishop et al. (2000), Passarinho and de Vries (2002), and Tiffen (2004).

Bayesian inference (BI) was used to build phylogeny for the angiosperm-wide dataset in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) under the WAG+I+G model of evolution as determined by the Bayesian information criterion (BIC) in ProtTest v2.4.mac (Abascal et al. 2005). Two Bayesian analyses were performed simultaneously with posterior probabilities (pp) of the generated trees approximated using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) algorithm with four incrementally heated chains for 2,132,000 generations while sampling trees every 100 generations until both analyses converged on similar log likelihood scores (average standard deviation of split frequencies <0.01). Data was further analyzed to ensure convergence with Tracer v1.5 (Rambaut and Drummond 2003-2009). SumTrees v3.0.0 (Sukumaran and Holder 2010) was used to combine the trees from both runs using a burn-in of 2,132 trees per run and to assemble a 50% majority rule tree from the remaining trees.

Under the Blosum62+I+G+F model of evolution as determined by BIC in ProtTest, two Bayesian analyses were performed for the HMM-derived dataset simultaneously with posterior probabilities of the generated trees approximated using the MCMCMC algorithm with four incrementally heated chains for 1,782,000 generations, sampling trees every 100 generations. Similar to the phylogenetic analyses of angiosperm-wide dataset, analyses were run until log likelihood scores converged as determined by visual inspection of runs with Tracer. SumTrees was used to combine trees from both runs following removal of a burn-in of 1,782 trees per run and to assemble a 50% majority rule tree post burn-in. Angiosperm-wide and HMM-derived phylogenetic trees were edited in FigTree v1.3.1 and Adobe® Illustrator®.

Detecting selection

As an initial assessment of evolutionary forces acting on angiosperm class I chitinases, we used TestBranchdNdS method in HyPhy 2.0.mac (Pond et al. 2005) to test whether branches leading to the two major class I chitinase lineages evolved under different selection pressures than the remaining branches of the phylogeny (fig. 2.2). The analysis was performed using the with the MG94xGTR nucleotide substitution model, site-to-site rate variation model with dN (the number of non-synonymous substitutions per non-synonymous site) and dS (the number of synonymous substitutions per synonymous site) allowed to vary simultaneously, and the default amino acid class model. For lineage-specific tests, two evolutionary models were fit to the tree: (1) the rates of protein evolution are the same across the entire tree, and (2) the rates of protein evolution are different in the branch separating Class I-Clade 1 or Class I-Clade 2 from the rest of the class I and IV chitinases in the phylogeny (fig. 2.2). Because models 1 and 2 are nested, we used a likelihood ratio test (LRT) to determine statistical significance of improvement in likelihood with the addition of parameters in model 1.

To further test certain lineages for differential selection, we used the fixed effects likelihood (FEL) model in HyPhy 2.0.mac to estimate non-neutral evolution for specific branches of the BI 50% majority rule consensus tree for the HMM-derived dataset (fig. 2.2). For each lineage, a two-rate analysis was used to allow adjustment of dN and dS across sites, a GTR model crossed with MG94 was specified for the nucleotide model of evolution, and dN/dS was estimated from the data with branch corrections. As suggested by the HyPhy program default, p -values < 0.10 were considered to be significant.

Template identification, modeling homology, and functional analysis

Protein structure homology-modeling of full-length *N. khasiana* subclass Ia (NkChitI-1) and Ib (NkChitI-3) chitinases was performed using gapped blast and HHSearch database searches with the SWISS-MODEL server (Peitsch 1995; Arnold et al. 2006; Kiefer et al. 2009) against HMMPfam, HMMTigr, ProfileScan, SuperFamily libraries. A crystal structure of a family 19 chitinase from *Carica papaya* (PDB ID: 3cqlB) was identified as the closest homolog to NkChitI-1 and NkChitI-3 in complex with NAG (Bernstein et al. 1977). Comparative modeling of *N. khasiana* class I chitinase was accomplished by constructing a model of NkChitI-1 and NkChitI-2 from *C. papaya* 3cqlB utilizing the SWISS-MODEL alignment mode tool. The program MacPyMOL v1.3 (Schrödinger LLC) was then used to thread the three-dimensional models of NkChitI-1 and NkChitI-2 to 3cqlB associated with two NAG oligomers in the active site cleft and introduced water molecules (Huet et al. 2008). To detect substrate interacting regions, polar contacts ≤ 5 Å were visualized in MacPyMOL between *N. khasiana* subclass Ia/Ib chitinases and 3cqlB-complexed NAG and water molecules positioned in the active site cleft via threading. Sites identified as under positive selection by the two-rate FEL model that coincide with residues directly interacting with NAG or in water-mediated interactions were mapped to PDB coordinates in MacPyMOL.

2.3 Results

Class I chitinase homologs expanded in land plant genomes

Within the land plant genomes studied here, we were unable to identify genes that contained all required class I chitinase signatures from *P. patens* (a moss) and *S. moellendorffii* (a lycophyte) genomes. Instead, chitinases recovered from these taxa lacked a chitin-binding site characteristic of class I.

Subclass Ia and Ib chitinases distinguished by the presence or absence of a CTE and are represented generally within eudicot genomes, however when considering any individual genome, the presence of both subclasses is not guaranteed (fig. 2.1A-B, table 2.2). Similar to a previous analysis of the *A. thaliana* chitinase gene family (Passarinho and de Vries 2002), we found a single subclass Ia chitinase with a 7-residue-long CTE (AtChitI-1). On the contrary, one subclass Ib chitinase was recovered from the *V. vinifera* genome (VvChitI-1), which is missing a CTE indicative of extracellular activity, a portion of chitin-binding domain, and the majority of the proline-rich hinge.

Subclass Ib chitinases of *G. max* (GmChitI-1 and GmChitI-2) and *M. trunculata* (MtChitI-1) are missing a CTE like that of VvChitI-1, but have a complete proline-rich hinge. *L. japonicus* LjChitI-1 is missing a portion of the proline-rich hinge similar to GmChitI-1, GmChitI-2, and MtChitI-1, yet unlike these Fabaceae class I chitinases, it is subtended by a 9-residue-long CTE; an indicator that LjChitI-1 is a subclass Ia chitinase. Residues identified as important players in catalytic activity and substrate binding (Garcia-Casado et al. 1998) are conserved in GmChitI-1, GmChitI-2, MtChitI-1, and LjChitI-1 with the exception of Thr273 located in active site IV of the catalytic domain.

In *P. trichocarpa*, PtChitI-1, PtChitI-2, PtChitI-3, PtChitI-4, PtChitI-5 are all subclass Ia chitinases based on the presence of a CTE. The CTE is relatively conserved between PtChitI-1, PtChitI-2, PtChitI-3, PtChitI-4, beginning with Gly-Leu-Leu, however the C-terminal extension of PtChitI-5 is deviant, beginning with Thr-Leu-Gly and containing an extra residue (10 residues in PtChitI-5 versus 9 residues in PtChitI-1, PtChitI-2, PtChitI-3, and PtChitI-4). All are missing portions of the proline-rich hinge, and 6+ glycine repeats can be found in this domain for PtChitI-1, PtChitI-3, and PtChitI-5. Similar glycine repeats are present in PtChitI-2 and PtChitI-4 in the proline-rich hinge domain, but to less of a degree. A number of amino acid substitutions are present in the active sites of the catalytic domain, namely, in active site III where tyrosine (Tyr256) has been substituted for a tryptophan at a site identified to participate in substrate binding (Garcia-Casado et al. 1998).

Like eudicot genomes, monocot genomes contain both subclasses Ia and Ib chitinases (fig. 2.1A-B, table 2.2). Of the 4 class I chitinases identified in the *O. sativa* ssp. *japonica* genome, only one (OsChitI-2) is considered a subclass Ia chitinase based on the presence of a 9-residue-long CTE (beginning with Gly-Ser-Ser). OsChitI-3 is unique in that threonine (Thr273) is substituted for serine at a site demonstrated to bind directly to the substrate (Brameld and Goddard 1998; Fukamizo 2000; Huet et al. 2008), while OsChitI-1, OsChitI-2, and OsChitI-4 all have the conserved serine (Ser273). Also within active site IV of OsChitI-1 and OsChitI-2, phenylalanine (Phe276) has been substituted for tyrosine at a site identified as important for catalytic activity and substrate binding (Verburg and Huynh 1991; Verburg et al. 1992; Verburg et al. 1993; Huet et al. 2008).

We found two class I chitinases in the *Z. mays* ssp. *mays* genome (ZmChitI-1 and ZmChitI-2), both of which are identified as subclass Ia and vacuolar based on the presence of CTE extensions, 9 and 16 residues respectively. Each *Z. mays* ssp. *mays* class I chitinase has a proline-rich hinge with glycine repeats. Additionally, in ZmChitI-1 at Arg315, arginine is substituted for glutamine, a residue important in substrate binding (Garcia-Casado et al. 1998). The CTE of ZmChitI-1 and ZmChitI-2 are highly divergent from each other and do not begin with Gly-Leu-Leu as in AtChitI-1. Tiffen's (2004) molecular evolutionary study of chitinase gene family members in Poaceae included subclass Ib chitinase genes amplified from *Z. mays* ssp. *parviglumis* and *Z. diploperennis*, yet we were unable to recover class I chitinases without a C-terminal extension from the *Z. mays* ssp. *mays* genome.

The majority of the proline-rich hinge of *S. bicolor* class I chitinases is missing, with only two proline repeats in this domain for SbChitI-1. SbChitI-1 has a CTE (subclass Ia) while SbChitI-2 does not (subclass Ib). As in ZmChitI-1, an arginine is substituted for glutamine at Arg315 in SbChitI-1.

The *B. distachyon* genome contains subclass Ia and Ib chitinases, all of which have the proline-rich hinge domain: BdChitI-1, BdChitI-2, and BdChitI-3 are all missing a CTE (likely subclass Ib), whereas BdChitI-4 has an exceptionally long +41 residue CTE (subclass Ia). BdChitI-4 is also missing a region 5-prime to active site VII in the catalytic domain and has a single amino acid change from serine to threonine (Thr273) in active site IV a site observed to form a direct hydrogen bond with NAG (Brameld and Goddard 1998; Huet et al. 2008).

Class I chitinase subclasses are identified in carnivorous plants and related genera of the Caryophyllales

Comparative analyses of class I chitinases amplified from *Nepenthes*, *Drosera*, and *Dionaea*, *Ancistrocladus*, and *Triphyophyllum* show differences in the length and sequence of non-coding regions (see GenBank for gDNA sequences, table 2.1), while coding regions are fairly conserved at the amino acid level within respective subclasses as determined via pairwise distance. Subclasses are reciprocally monophyletic, indicating origins prior to the evolution of the carnivorous Caryophyllales (fig. 2.1A).

Four *Nepenthes* sequences were recovered from GenBank (NkChitI-1, NkChitI-2, NkChitI-3, NkChitI-4) representing both subclass Ia and Ib chitinases (table 1). Subclass Ia chitinases (NkChitI-1 and NkChitI-2) possess a CTE beginning with Gly-Leu-Leu, comparable to the vacuolar eudicot class I chitinases, and are characterized as constitutively expressed housekeeping chitinases (Eilenberg et al. 2006). Similar to OsChitI-1 and OsChitI-2, both possess a phenylalanine at site 276 in active site IV of the catalytic domain. NkChitI-1 and NkChitI-2 are also missing the majority of the proline-rich hinge domain. Subclass Ib chitinases (NkChitI-3 and NkChitI-4) are both missing a CTE and are secreted into the pitcher fluid when induced in response to chitin (Eilenberg et al. 2006). NkChitI-3 and NkChitI-4 also have the proline-rich hinge, similar to the monocot class I chitinases. Unlike NkChitI-1 and NkChitI-2, NkChitI-3 and NkChitI-4 do not have the phenylalanine substitution in active site IV.

We sequenced subclass Ia and Ib chitinases from *N. mirabilis* and *N. maxima* (fig. 2.1A-B, table 2.1), categorizing them based on percent pairwise identity of translated CDS sequences to previously-sequenced *N. khasiana* class I chitinases. NmaxChitI-1, NmaxChitI-2 and NmirChitI-1 share high percent similarity with NkChitI-1 and NkChitI-2 (>99%), suggesting that these

sequences are subclass Ia chitinases. NmirChitI-3 shows high percent similarity (>99%) with NkChitI-3 and NkChitI-4 and could be a subclass Ib chitinase. Two additional sequences were recovered from *N. mirabilis* and *N. maxima* with domains similar to *N. khasiana* subclass Ib chitinases. Although these sequences have the proline-rich hinge characteristic to NkChitI-3 and NkChitI-4, they also contain stop codons and/or a base pair change that results in disruption of the conserved H-E-T-T motif. We consider these possible pseudogenes, as premature stop codons would hinder translation into a full-length class I chitinases. Our phylogenetic analyses exclude *N. mirabilis* and *N. maxima* class I chitinase pseudogenes, but sequences were submitted to GenBank (JN867627 and JN867630).

Subclass Ia and Ib chitinases were retrieved for taxa within the genus *Drosera* either via GenBank queries or by PCR (fig. 2.1A-B, table 2.1). A single class I chitinase has been previously sequenced from *D. rotundifolia* (DrChitI-1). The CDS translation of DrChitI-1 has high percent identity to NkChitI-1 and NkChitI-2 (93.1%), which suggests that this may be a subclass Ia chitinase. We amplified two additional *D. rotundifolia* chitinase gene sequences (DrChitI-2 and DrChitI-3), one of which includes part of the proline-rich hinge domain, and both of which contain the 5-prime region of the catalytic domain with valine, as in the monocot class I chitinases described here. DrChitI-2 and DrChitI-3 share high percent identity with NkChitI-3 and NkChitI-4, and may be subclass Ib sequences. DsChitI-1 is a previously sequenced *D. spathulata* class I chitinase, which has high percent identity (91.2%) with NkChitI-3 and NkChitI-4 and therefore could be subclass Ib sequence. A notable deviation for DsChitI-1 is a substitution of tryptophan for a tyrosine (Tyr256) in active site III, a site identified as important in substrate binding (Garcia-Casado et al. 1998). We amplified a region corresponding to a region of the catalytic domain from two *D. binata* chitinases. Both share high percent identity with NkChitI-3 and NkChitI-4 and therefore may be subclass Ib sequences. A partial chitinase gene sequence corresponding to a region of the proline-rich hinge and the catalytic domain was amplified from *D. capensis* (DcChitI-1). As the CDS translation shares high percent identity (94.0%) with NkChitI-3 and NkChitI-4, this could be subclass Ib sequence. Like DrChitI-2, the 5-prime end of the catalytic region is preceded by a string of 3 glycines. Valine is also present in the 5-prime end of the catalytic region, similar to DrChitI-2 and DrChitI-3 and monocot class I chitinases described here. We were able to extend amplification to the H-E-T-T motif for DcChitI-1, another indicator of this gene sequence's homology with class I chitinases.

Two partial *D. muscipula* chitinase sequences were also included in our analyses (fig. 2.1A-B, table 2.1). These comprise DmChitI-1, a chitinase amplified by PCR and DmChitI-2, a class I chitinase mRNA sequence previously deposited into GenBank (AY643484). The CDS translation of DmChitI-1 begins just after the start of the catalytic domain, extends through the H-E-T-T motif, and terminates after active site VII. Similar to NkChitI-1 and NkChitI-2, tyrosine is substituted with phenylalanine in active site IV. DmChitI-1 shares moderately high percent identity (89.6%) with NkChitI-1 and NkChitI-2, and is possibly a subclass Ia chitinase. The translated CDS of DmChitI-2 begins within active site IV and extends through active site VI. Interestingly, and quite unlike all the other class I chitinases described here, active site IV has a cysteine at site 276 (Cys276). DmChitI-2 shares 94.0% identical sites with NkChitI-3 and NkChitI-4 and thus may be a subclass Ib chitinase.

We amplified five partial chitinase genes from two species of *Ancistrocladus*: *A. grandiflorus* and *A. robertsoniorum* (fig. 2.1A-B, table 2.1). Compared to class I chitinases amplified from *Nepenthes*, *Ancistrocladus* amplicons corresponding to the five chitinases described were unusually short (<500bp). The CDS translation of AgChitI-1 contains all seven active sites of the catalytic domain, the first active site having the conserved H-E-T-T motif. A portion of the proline-rich hinge is

present in the AgChitI-2 sequence, as well as the 5-prime region of the catalytic domain up to the first active site of the catalytic domain. AgChitI-3 contains the same region as AgChitI-2, but differences are found in the proline-rich hinge. We were able to partially sequence ArChitI-1 to include the 5-prime region of the catalytic domain through active site I, as well as amplify a portion of ArChitI-2 from the proline-rich hinge up to the first active site of the catalytic domain. Considerable nucleotide differences within the first and second intergenic regions can be found between ArChitI-1 and ArChitI-2. AgChitI-1 and ArChitI-1 share high sequence identity with NkChitI-1 and NkChitI-2, and may be subclass Ia chitinases. The sequence identity shared between AgChitI-2 and ArChitI-2 and full-length *N. khasiana* class I chitinases is almost identical, making it difficult to classify these sequences on site similarity alone. AgChitI-3 shares high sequence identity (92.4%) with NkChitI-1 and NkChitI-2, yet phylogenetic analyses place AgChitI-3 close to NkChitI-3 and NkChitI-4 (fig. 2.1A and 2.2).

Similar to the *Ancistrocladus* class I chitinases described above, certain amplified chitinase PCR products from *Triphyophyllum* were shorter in length than expected: the CDS for TpChitI-3 is ~200bp, whereas TpChitI-1 and TpChitI-2 range from ~500-800bp. TpChitI-1 and TpChitI-3 do not span the proline-rich hinge, but begin either at the 5-prime end of the catalytic domain (TpChitI-2) or at active site I (TpChitI-1). TpChitI-1 and TpChitI-2 extend to the sixth active site, and TpChitI-2 continues to include active site VII.

Evolution of class I chitinases in 15 angiosperm genera

Phylogenetic analysis of class I chitinases indicates there are three major lineages of class I chitinases in the angiosperms (fig. 2.1A). The first lineage (A) includes vacuolar-located (subclass Ia) and extracellular (subclass Ib) class I chitinases of the eudicots. Within this lineage, there are three distinct clades. One well-supported (0.99 pp) clade (A1) is composed of Caryophyllales-exclusive (*Ancistrocladus*, *Nepenthes*, and *Triphyophyllum*) class I chitinases, two of which are known to have a CTE (*N. khasiana*, NkChitI-1 and NkChitI-2). The second clade (A2) includes *A. thaliana*, *L. japonicus*, and *P. trichocarpa* class I chitinases, all of which have a CTE. A single carnivorous plant class I chitinase (*D. rotundifolia*, ChitI-2) is positioned sister to this clade. Five additional eudicot class I chitinases comprise the third clade (A3), which includes a partial class I chitinase from *D. muscipula* and full-length *G. max*, *M. truncatula*, and *V. vinifera* sequences without a CTE.

A second major lineage sister to lineage A is yet another Caryophyllales-exclusive clade that is relatively well-supported (0.86 pp) (B). Two clades are present in this lineage. The first clade (B1) is exclusive to *Nepenthes* class I chitinases, two of which (*N. khasiana*, NkChitI-3 and NkChitI-4) are full-length sequences which are missing a CTE. The second clade (B2) is a polytomy of partially sequenced *Ancistrocladus*, *Dionaea*, *Drosera*, and *Triphyophyllum* class I chitinases.

The monocot class I chitinases form another lineage (C) with good support (0.76 pp). A few phylogenetic relationships can be inferred; *B. distachyon* class I chitinases with and without a CTE (BdChitI-4 and BdChitI-1 through 3, respectively) form a monophyletic group (C1, 0.98 pp), while *O. sativa* ssp. *japonica*, *S. bicolor*, and *Z. mays* class I chitinases are polyphyletic. Clade C2 (0.98 pp) contains members with (*ZmChitI-2*) and without (*OsChitI-1*, *OsChitI-4*, *OsChitI-3*, and *SbChitI-2*) a CTE, whereas clade C3 (1.00 pp) includes *O. sativa* ssp. *japonica*, *S. bicolor*, *Z. mays* ssp. *mays* class I chitinases all with a CTE (*OsChitI-2*, *SbChitI-1*, and *ZmChitI-1*).

In the phylogenetic analysis of HMM-derived class I chitinases from *A. thaliana*, *O. sativa* ssp. *japonica*, *S. bicolor*, and *V. vinifera* genomes, two major lineages are present (fig. 2.2). The first

lineage (Class I-Clade 1) includes two subfamilies of Caryophyllales class I chitinases. The first clade (I-4) contains *Nepenthes* class I chitinases, two of which are known to have a CTE (*N. khasiana*, NkChitI-1 and NkChitI-2). Sister to this clade is a single *T. peltatum* class I chitinase (TpChitI-2). The second clade (I-6) is comprised of *Ancistrocladus* and *T. peltatum* class I chitinases. One *Drosera* class I chitinase (DrChitI-1) is sister to the two described clades of *Ancistrocladus*, *Nepenthes*, and *Triphyophyllum* chitinases (I-2). A single *V. vinifera* class I chitinase (VvChitI-1) without a CTE is placed at the base of Class I-Clade 1 (1-1).

The second major lineage of class I chitinases (fig. 2.2, Class I-Clade 2), includes eudicot and monocot class I chitinases. Three clades are present in Class I-Clade 2, the first (I-8, 0.93 pp) contains *O. sativa* ssp. *japonica* and *S. bicolor* class I chitinases without a CTE (OsChitI-1, OsChitI-3, OsChitI-4, and SbChitI-2). The second clade (1-10, 1.00 pp) contains only *Nepenthes* class I chitinases, two of which are full-length sequences (*N. khasiana*, NkChitI-3 and NkChitI-4) that are missing a CTE. Clade I-11 is a polytomy containing *Ancistrocladus*, *Dionaea*, *Drosera*, and *Triphyophyllum* class I chitinases (0.84 pp).

Certain class I chitinase lineages and sites carry different signatures of selection

The TestBranchdNdS test in HyPhy indicated that dN/dS is significantly different in Class I-Clade 1 ($p = 0$) as compared to the rest of the phylogeny (fig. 2.2). Conversely, dN/dS in Class I-Clade 2 is not significantly different ($p = 0.22539$) than dN/dS for the remainder of the tree.

To further estimate non-neutral evolution for specific branches of the HMM-derived class I chitinase phylogeny, we used the FEL method in HyPhy. Sites identified by the FEL method as being under positive selection are found in both major lineages of the class I chitinases (Class I-Clade I and Class I-Clade 2, fig. 2.3, and table 2.3). In Class I-Clade 1, clades I-2, I-4, and I-5 each contain at least one site under positive selection that falls within one of the seven active sites (see A.5). In clade I-5, which contains *Nepenthes* subclass Ia chitinases, site 276 falling within active site IV is identified as under positive selection. In Class I-Clade 2, the FEL method identified site 317 positioned within active site V as under positive selection in clade I-9. Amino acid site 156, a positively selected site detected by the FEL method, is shared between the two major lineages of class I chitinases in clades I-2 (Caryophyllales subclass Ia), and I-3 (Caryophyllales subclass Ia, excluding DrChitI-1), I-4 (Caryophyllales subclass Ia, including TpChitI-2 and *Nepenthes* sequences), I-7 (Class I-Clade 2 in its entirety), and I-8 (*O. sativa* ssp. *japonica* and *S. bicolor* subclass Ib).

Substitutions in the active site of class I chitinases may affect interactions with chitin substrate

Homology-modeling revealed polar interactions between *N. khasiana* subclass Ia and Ib chitinase residues and NAG oligomers, some of which involve water molecules (fig. 2.4). In subclass Ia (NkChitI-1) and Ib (NkChitI-3) chitinases, hydrogen bonding is observed between NAG and residues His214, Asn277, Ile352, Glu357, Arg365, and Arg370. Hydrogen bonding mediated by water molecules is also observed between NAG and NkChitI-1 residues Thr217, Asn350, Ile351, Asn353, Gly354, Gly355, Val356. In NkChitI-3, water bridges residues Thr217, Tyr276, Asn350, Ile351, Asn353, Gly354, Gly355, and Leu356 to NAG. A number of NkChitI-1 and NkChitI-3

residues directly interact with NAG, while also taking part in water-mediated hydrogen bonding. Residues that participate in this type of interaction are Glu215, Gln239, Gln271, Ser273, Tyr274, and Lys318 in NkChitI-1, and Glu215, Val239, Gln271, Ser 273, Tyr274, and Lys318 in NkChitI-3.

Three sites have the potential to influence hydrogen bonding and ultimately functionality due to residue discrepancies between NkChitI-1 and NkChitI-3 (fig. 2.4). At site 239, a glutamine is present in NkChitI-1 (Gln239), whereas a valine occupies the same site in NkChitI-3 (Val239). This site is positionally homologous to Arg90 in *C. papaya* GH family 19 chitinase, a residue involved in substrate binding that is also conserved across other plant GH family 19 chitinases (Huet et al. 2008). Site 276, identified as under positive selection in *Nepenthes* subclass Ia chitinases by the FEL method (clades I-4 and I-5, fig. 2.2, fig. 2.3, and table 2.3), is homologous in its position to a tyrosine residue in NkChitI-3 involved in water-mediated hydrogen bonding to NAG (fig. 2.4). In NkChitI-1, a phenylalanine occupies site 276, a substitution that prevents hydrogen bond formation due to the hydrophobic nature of the benzyl side chain. Lastly, NAG-interacting site 356 differs between NkChitI-1 and NkChitI-3 by the presence of either a valine or leucine, respectively. This site is homologous to *C. papaya* family 19 chitinase Leu202, a residue that forms a hydrogen bond with NAG by involving a water molecule (Huet et al. 2008).

2.4 Discussion

Molecular evolution of class I chitinases in angiosperms

While phylogenetic relationships among chitinase gene families have been explored in monocot and eudicot taxa (Davis et al., 1991; Hamel et al., 1997; Tiffen, 2004; Bishop et al., 2006; Shores et al., 2008; Xu et al., 2007; Prakash et al. 2010; Rottloff et al., 2011), this study is the first to conduct an in-depth analysis of angiosperm class I chitinases, while focusing on the functional evolution of class I chitinase subclasses in the carnivorous plants of the Caryophyllales.

Phylogenetic reconstructions of class I chitinase homologs reveal three major lineages in the angiosperms and two well-supported clades containing either Caryophyllales subclass Ia or Ib chitinases (fig. 2.1). Sequences amplified from carnivorous genera *Dionaea*, *Drosera*, and *Nepenthes*, part-time carnivore *Triphyophyllum*, and non-carnivorous *Ancistrocladus* fall within subclass Ia and Ib chitinase specific clades, with the exception of DmChitI-1, a *D. muscipula* class I chitinase sequence that is unresolved in the HMM-derived phylogeny (fig. 2.2). Clade specificity for subclass (Ia, Ib) is inconsistent among other angiosperms class I chitinases analyzed. For example, *B. distachyon*, *O. sativa* ssp. *japonica*, *S. bicolor*, and *Z. mays* class I chitinases do not show phylogenetic affinity based on the presence or absence of a CTE. Additionally, the eudicot subfamily containing *G. max*, *V. vinifera*, and *M. truncatula* chitinases without a CTE do not fall within the lineage containing Caryophyllales subclass Ib chitinases. Therefore, with the exception of subclasses retrieved from the Caryophyllales, a well-supported division is relatively absent between subclasses of eudicot and monocot class I chitinases based on phylogenetic history. This observation is consistent with earlier studies of evolutionary relationships among flowering plant chitinases (Hamel et al. 1997; Xu et al. 2007), where class I chitinases clustered in clades based on taxonomic affinity rather than subclass.

The separation of Caryophyllales subclass Ia and Ib chitinases into two distinct clades could be an indicator of a subfunctionalization, an explanation for the preservation of duplicate copies introduced by Force et al. (1999) in which complementary, degenerative mutations in duplicates leads to persistence of each copy. As evidenced by the presence of subclass Ia and Ib in monocots

and other eudicots, a duplication event obviously occurred prior to the evolution of the Caryophyllales, but each copy may have become specialized to perform complementary functions in carnivorous plants. Differences in expression patterns within the carnivorous trap may have contributed to subclass specialization and initiation of processes that lead to functional diversification. This idea is consistent with expression studies in *Nepenthes*, where differential expression of subclass Ia and Ib within the trap has been demonstrated prior to and after mimicking prey capture (Eilenberg et al. 2006). Subclass Ia chitinases are considered housekeeping chitinases, as they are constitutively expressed in *Nepenthes* traps, whereas subclass Ib chitinases are synthesized in traps only after the traps have been exposed to chitin. A similar observation was made for *Drosera*, in which chitinase activity differed among tissue types, most strikingly in the secretory stalked and sessile glands of the lamina after chitin exposure (Matusíková et al. 2005; Libantová et al. 2009). It seems as though in the carnivorous plants of the Caryophyllales, subclass Ia clade is responsible for pathogenesis response and subclass Ib clade for carnivory, two functions essential for preservation of the carnivorous habit.

If subclass Ib genes have been co-opted for plant carnivory, their presence in species that have partially (*Triphyophyllum*) or completely lost (*Ancistrocladus*) the carnivorous habit is curious. Due to the unexpectedly short length of amplicons (see results), these genes may have been converted to pseudogenes after loss of functional domains. It is plausible that during the transition from a plant carnivore to a part-time or non-carnivorous plant, *Triphyophyllum* and *Ancistrocladus* lost certain enzymes required for plant carnivory. Loss of chitinase function in the Caryophyllales is supported by the occurrence of *Nepenthes* subclass Ib chitinase homologs with premature stop codons recovered via PCR (see results). Gene birth and death events are common occurrences during gene family evolution (Nei et al. 2000; Hua et al. 2011), and clearly *Nepenthes* is not immune to chitinase gene turnover. GH family 19 chitinases, which include class I, are thought to be under notorious levels of domain rearrangement and excision. A study of class I chitinases in flowering plants documented perfect direct nucleotide repeats in the chitin-binding domain and proline-rich hinge, indicating possible transposition events that could have resulted in domain excision and ultimately the formation of new classes of chitinases (Shinshi et al. 1990). Transposition events may also be detrimental for chitinase genes, as the removal can produce pseudogenes (Finnegan 1989). In *Ancistrocladus*, we found a trinucleotide perfect repeat (GTG-GTG-GTG) in AgChitI-2 and AgChitI-3, and a trinucleotide imperfect repeat (GTA-GTG-GTG) in ArChitI2 immediately following the proline-rich hinge. *Triphyophyllum* TpChitI-3 also has a trinucleotide imperfect repeat (CTG-GTG-GTG) immediately following the proline-rich hinge, terminating just prior to the first active site of the catalytic domain. The presence of these repeats could be attributed to transposition events as suggested by Shinshi (1990) and could also account for observable differences in sequence length, especially if an excision event resulted in domain hitchhiking and removal. As expression data for these specific sequences is currently unavailable, it is difficult to say whether these genes are likely to be pseudogenes because of transposition events.

Selection acting on subclasses of Caryophyllales class I chitinases

Bishop et al.'s (2000) analyses of *Arabidopsis* class I chitinases suggest that adaptive replacements are localized disproportionately in the active site cleft of the chitinase enzyme. This pattern of replacements was taken as evidence for rapid coevolutionary interactions, most likely between plant chitinases and chitinolytic inhibitors produced by pathogenic fungi. Yet another molecular evolutionary study of class I chitinases in Poaceae (grasses) found positively selected sites to not be significantly overrepresented in the active cleft and that the majority of sites identified are not shared with *Arabidopsis* (Tiffen 2004). These results imply that selective pressures acting on class I chitinases may be lineage specific. We expand class I chitinase homologs to investigate signatures of selection in the Caryophyllales carnivorous plant lineage.

Rate variation was observed among codons at branches nested within Class I-Clade 1 containing Caryophyllales subclass Ia homologs, and Class I-Clade 2 containing Caryophyllales Ib homologs. Replacements associated with rate variation were present throughout protein sequences analyzed, a number of which fall within one or more active sites coded for in the catalytic domain (A.5, table 2.3). In clades I-5 and I-9 (fig. 2.2), replacements occurred at amino acid sites previously demonstrated to participate in substrate-binding and/or catalytic activity (Verburg and Huynh 1991; Verburg et al. 1992; Verburg et al. 1993; Anderson et al. 1997; Brameld and Goddard 1998; Garcia-Casado et al. 1998; Tang et al. 2004). Site 276 is positively selected in *Nepenthes* subclass Ia chitinase homologs (clades I-4 and I-5, fig. 2.2) and positionally homologous to a tyrosine residue essential for substrate binding in the catalytic site, but not catalysis (Verburg et al. 1992; Verburg et al. 1993). Selective replacement of this tyrosine residue with an alternative residue such as phenylalanine proves to significantly reduce enzymatic activity for a variety of angiosperm class I chitinases, up to 100% loss of activity in *Zea* (Verburg et al. 1992; Verburg et al. 1993; Anderson et al. 1997; Tang et al. 2004). Fascinatingly, all *Nepenthes* subclass Ia chitinases analyzed here have phenylalanine at site 276, yet *in vivo* studies have confirmed their activity in the carnivorous trap of *N. khasiana* (Eilenberg et al. 2006). This is in contrast to the *Nepenthes* subclass Ib chitinase homologs we recovered, which all contain the conserved tyrosine at this site. Protein structure homology-modeling of *N. khasiana* class I chitinases also provides evidence for the significance of site 276 in forming polar interactions with NAG. In *N. khasiana* subclass Ib chitinase NkChitI-3, Tyr276 forms a hydrogen bond with NAG mediated by a water molecule, whereas a phenylalanine at site 276 prevents hydrogen bond formation (fig. 2.4). Based on this observation, the presence of certain residues in the active site cleft could thus be very important in determining how subclasses of class I chitinases interact with the substrate.

Five positively selected sites in Caryophyllales class I chitinases were previously identified as a target of selection in *Arabidopsis* and *Zea* class I chitinases (Bishop et al. 2000; Tiffen 2004) (table 2.3). However, only one of these shared sites, 247, falls within the active cleft, and is a replacement exclusive to Caryophyllales subclass Ia chitinase homologs (clade I-2). That the majority of positively selected sites are not shared between these groups of taxa may infer that adaptive responses to selective pressures are lineage specific. Our results also suggest that adaptive responses could be subclass specific, as sites identified under positive selection are not shared between Ia and Ib subclasses of Caryophyllales class I chitinases. Tiffen (2004) proposed that selection favors functional divergence of duplicated genes involved in pathogenic response, as plants are afflicted by a wide variety of pathogens. The fact that chitinases are well known for their substrate specificity and different antifungal abilities is indicative that having a number of defense mechanisms confers some selective advantage (Brunner et al. 1998; Schultze et al. 1998). It is thus reasonable that carnivorous

plant subclass Ib chitinases would have a signature of selection different from that of subclass Ia, as the absence of a CTE enables subclass Ib chitinases to escape from digestive glands into the carnivorous trap and access materials different from those found intracellularly (Adlassnig et al. 2011). Additionally, if positive selection has driven the evolution of Caryophyllales subclass Ia chitinases at sites important for functionality, then these enzymes may have evolved greater activity against fungal pathogens, whereas selection on Caryophyllales subclass Ib chitinases has been relaxed.

2.5 Conclusion

The utilization of digestive enzymes for the carnivorous habit can be extended to other members of the GH families. Class IV chitinases have been recently identified by proteome analysis to be present in non-exposed *N. alata* pitcher fluid (Hatano and Hamada 2008), while GH family 18 class III chitinases have been demonstrated to be up-regulated in the presence of prey in a few species of *Nepenthes* (Rottloff 2011). The occurrence of multiple classes of chitinase in the carnivorous trap may indicate synergistic roles in insect digestion, some of which could be influenced by differential expression patterns. Further molecular evolutionary studies of carnivorous plant digestive enzymes thus are warranted to explore functional divergence that can be promoted by shifts in selection.

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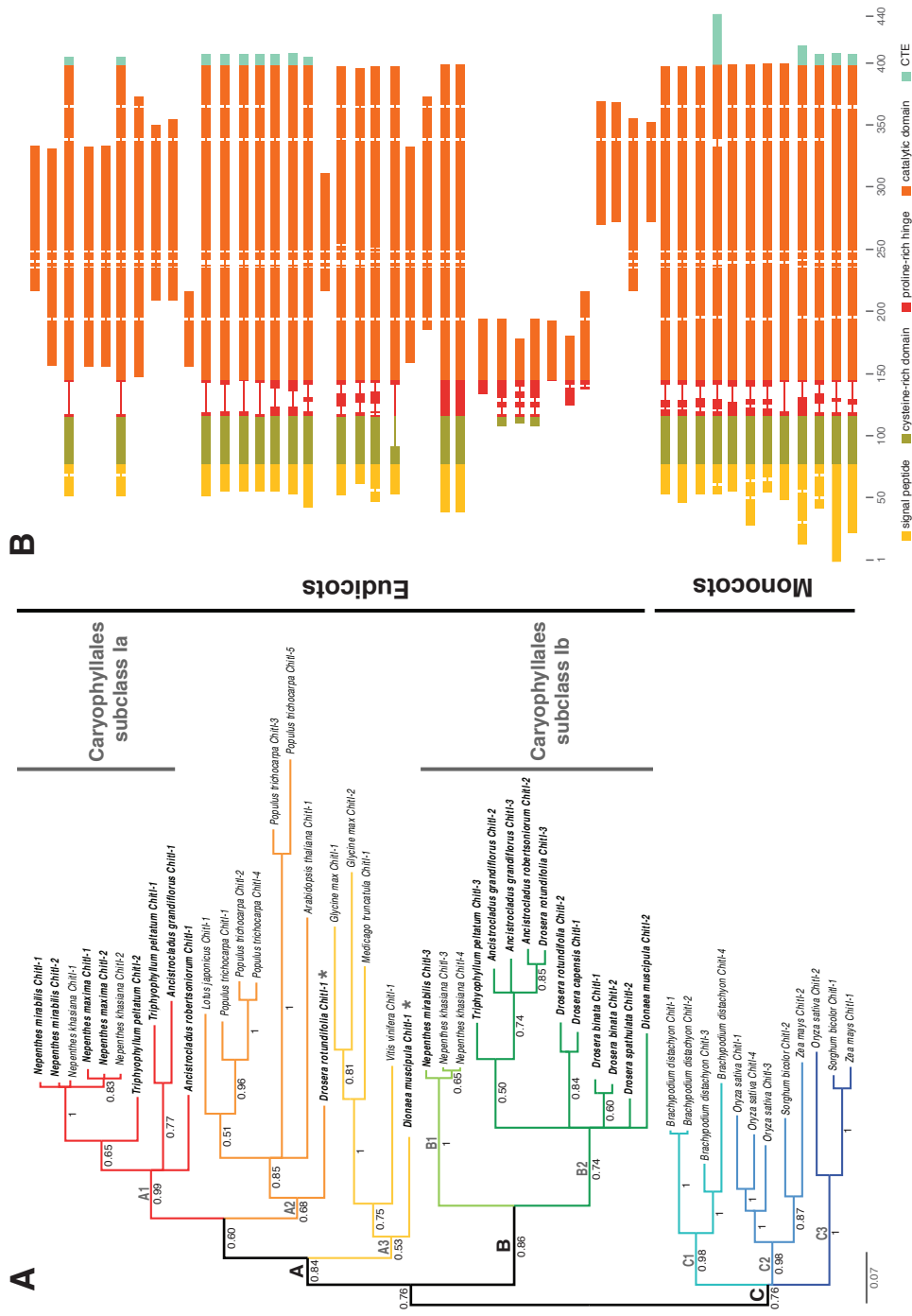


Fig. 2.1 Angiosperm-wide phylogenetic reconstruction for the class I chitinases. (A) Results from BI analysis of 49 class I chitinase homologs retrieved from angiosperms. Posterior probabilities (pp) from complete analysis are indicated at nodes on the Bayesian 50% majority rule tree. Chitinases in bold indicate partial sequences obtained from GenBank or amplified via PCR and asterisks mark *Dionaea* and *Dioscorea* sequences that do not fall within Caryophyllales subclass Ia or Ib specific clades. (B) Representation of class I chitinase homologs and their consecutive domains deduced from the multiple sequence alignment available in A.5.

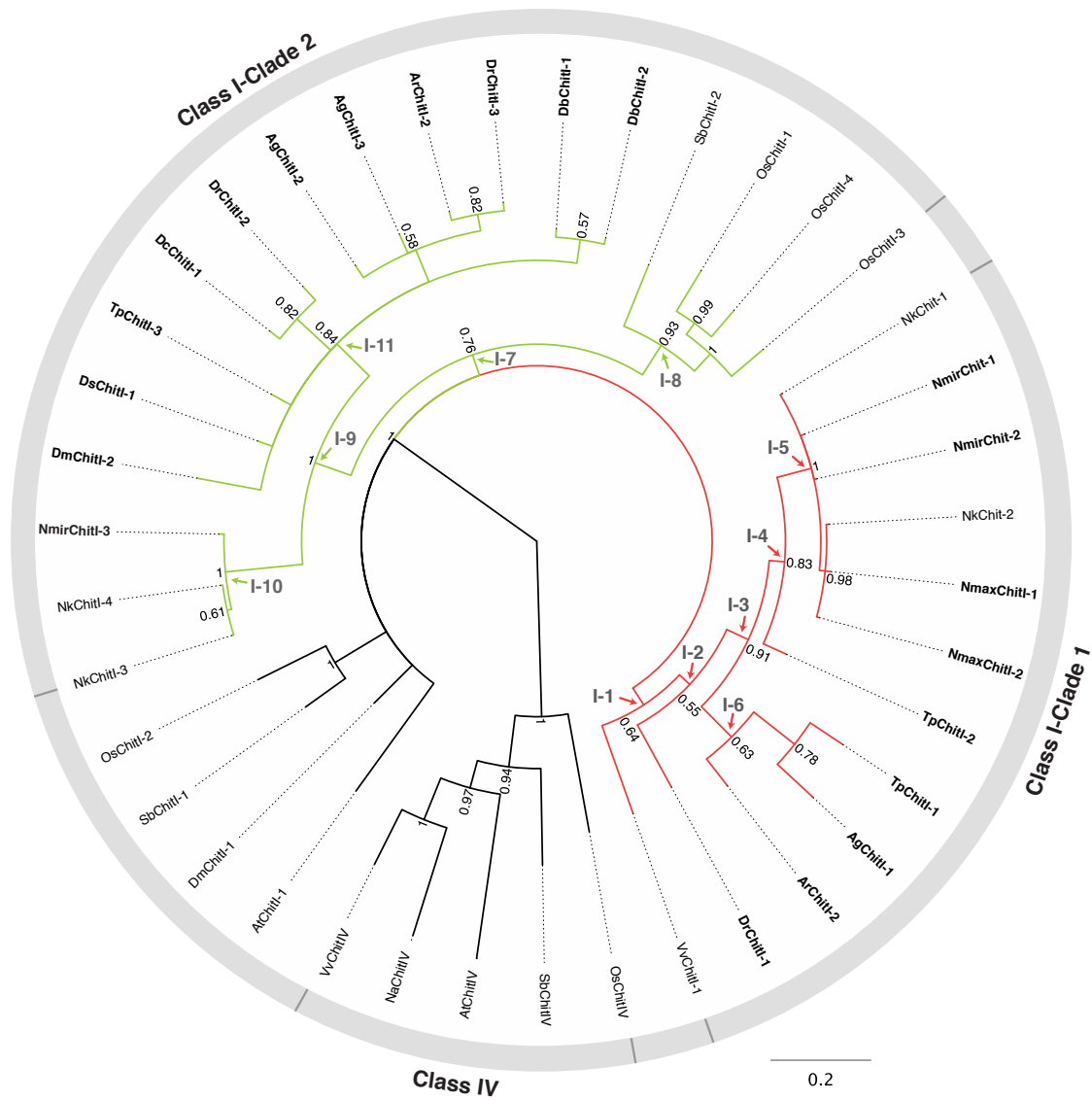


Fig. 2.2 HMM-derived phylogenetic reconstruction for the carnivorous Caryophyllales. Results from BI analyses of the HMM-derived dataset of class I chitinase homologs. Posterior probabilities (pp) are indicated at nodes on the Bayesian 50% majority rule tree. Chitinases in bold indicate partial sequences obtained from GenBank or amplified via PCR. Marked arrows indicate lineages tested for instances of differential selection by FEL. Chitinases in bold indicate partial sequences obtained from GenBank or amplified via PCR.

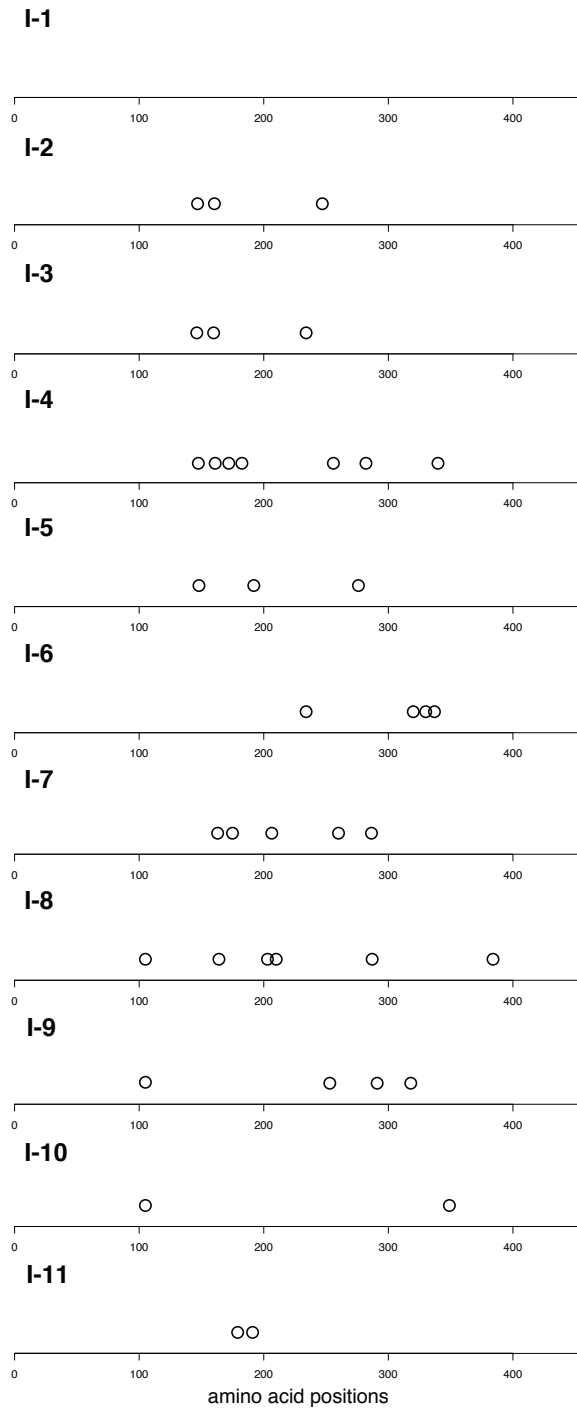


Fig. 2.3 Distribution of positive selected sites along translated CDS of the class I chitinase genes. Sites under positive selection identified by the two-rate FEL model in HyPhy (circles, $p < 0.1$) in clades I-1 through I-11 of fig. 2.2.

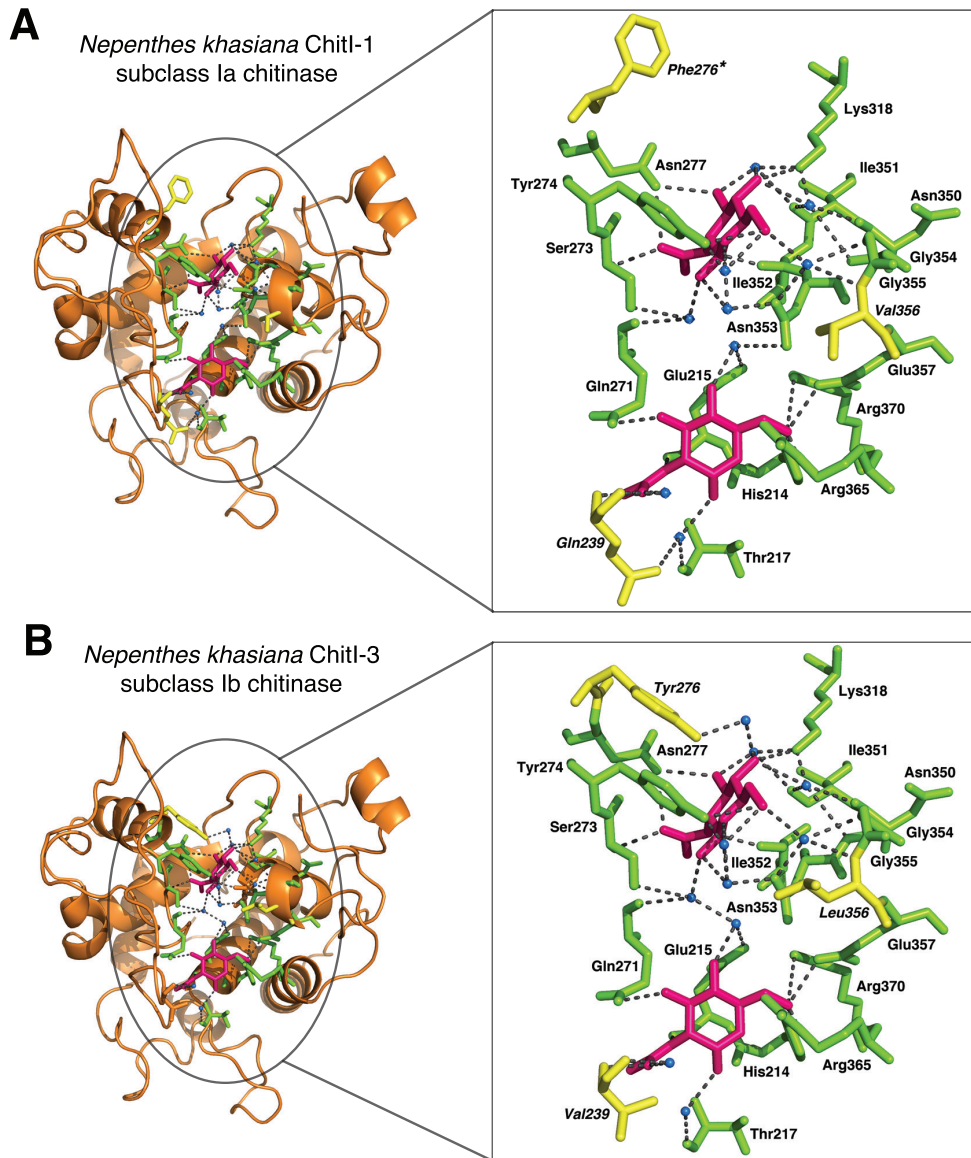


Fig. 2.4 Homology-modeling of *Nepenthes khasiana* subclass Ia and Ib chitinases. 3D models of (A) subclass Ia chitinase NkChitI-1 and (B) subclass Ib chitinase NkChitI-3 in association with NAG and water. Expansion of the active site cleft (right) depicts substrate-interacting residues (green and yellow) with polar contacts ≤ 5 Å to two NAG oligomers (pink) and introduced water molecules (blue). Sites that differ in the residue type interacting with the substrate are shown in yellow and are italicized. In (A), residue Phe276 (italicized and asterisk) disrupts the formation of a water-mediated hydrogen bond to NAG. Site 276 is positively selected in *Nepenthes* subclass Ia chitinase homologs (clade I-5, fig. 2.2) as identified by the two-rate FEL model in HyPhy.

Table 2.1 Caryophyllales class I chitinases

Class I Chitinase	GenBank ID
<i>Ancistrocladus grandiflorus</i>	
AgChitI-1	JN867619
AgChitI-2	JN867620
AgChitI-3	-
<i>Ancistrocladus robertsoniorum</i>	
ArChitI-1	JN867621
ArChitI-2	JN867622
<i>Dionaea muscipula</i>	
DmChitI-1	JN867626
DmChitI-2	AY643484
<i>Drosera binata</i>	
DbChitI-1	JN867623
DbChitI-2	JN867624
<i>Drosera capensis</i>	
DcChitI-1	JN867625
<i>Drosera rotundifolia</i>	
DrChitI-1	AY622818
DrChitI-2	-
DrChitI-3	-
<i>Drosera spatulata</i>	
DsChitI-1	AY643483
<i>Nepenthes khasiana</i>	
NkChitI-1	AY618885
NkChitI-2	AY618887
NkChitI-3	AY618881
NkChitI-4	AY618883
<i>Nepenthes maxima</i>	
NmaxChitI-1	JN867628
NmaxChitI-2	JN867629
<i>Nepenthes mirabilis</i>	
NmirChitI-1	JN867631
NmirChitI-2	JN867632
NmirChitI-3	JN867633
<i>Drosera rotundifolia</i>	
DrChitI-1	AY622818
DrChitI-2	-
DrChitI-3	-
<i>Triphyophyllum peltatum</i>	
TpChitI-1	JN867634
TpChitI-2	JN867635
TpChitI-3	-

Dashes represent class I chitinase sequences >200bp that are available in A.5.

Table 2.2 Class I chitinases identified by homology-based searches of angiosperm genomes

Genome location	Gene ID
<i>Arabidopsis thaliana</i>	
AtChitI-1 3: 3962382 - 3963984	AT3G12500
<i>Brachypodium distachyon</i>	
BdChitI-1 1: 25385703-25386665	Bradi1g29887.1
BdChitI-2 1: 25351801-25352859	Bradi1g29890.1
BdChitI-3 2: 47548066-47549245	Bradi2g47210.1
BdChitI-4 2: 47539862-47541277	Bradi2g47190.1
<i>Glycine max</i>	
GmChitI-1 2: 3943354-3946663	Glyma02g04820.2
GmChitI-2 16: 26279746-26281120	Glyma16g22680.1
<i>Lotus japonicus</i>	
LjChitI-1 AP004945: 3792-4848	AP004945_25
<i>Medicago truncatula</i>	
MtChitI-1 8: 19907150-19909682	Medtr8g074350.1
<i>Oryza sativa</i> ssp. <i>japonica</i>	
OsChitI-1 3: 17389600-17390681	LOC_Os03g30470
OsChitI-2 5: 19363900-19365359	LOC_Os05g33130
OsChitI-3 6: 30886217-30887473	LOC_Os06g51050
OsChitI-4 6: 30889813 - 30890862	LOC_Os06g51060
<i>Populus trichocarpa</i>	
PtChitI-1 scaffold_4: 18602513-18603761	POPTR_0004s18870.1
PtChitI-2 scaffold_9: 11302400-11303661	POPTR_0009s14420.1
PtChitI-3 scaffold_9: 11305831-11307159	POPTR_0009s14430.1
PtChitI-4 scaffold_9: 11275939-11277215	POPTR_0009s14380.1
PtChitI-5 scaffold_9: 11279620-11280968	POPTR_0009s14390.1
<i>Sorghum bicolor</i>	
SbChitI-1 9: 48498207-48499444	Sb09g019660
SbChitI-2 9: 1068140-1069105	Sb09g001210
<i>Vitis vinifera</i>	
VvChitI-1 chr4_random: 51200-52868	GSVIVT01007190001
<i>Zea mays</i> ssp. <i>mays</i>	
ZmChitI-1 8: 88706159-88706973	GRMZM2G062974 T01
ZmChitI-2 6: 82813531-82815309	GRMZM2G145518 T01

Class I chitinase; ID. Genome location refers to the chromosomal location.

Table 2.3 Sites identified as under positive selection by the two-rate FEL model

Clade	Amino Acid Site ^a	dN/dS	<i>p</i> -value
Class I-Clade 1			
I-1	-	-	-
I-2	151 ^f	291.866	0.00821021
	156	214.846	0.0694136
	247 ^{d,g}	21230.7	0.0668876
I-3	151 ^g	64.7185	0.00871179
	156	41.1455	0.0906384
	234	752463	0.000479813
I-4	151 ^f	398.085	0.008519
	156	246.081	0.0679215
	169	63.6414	0.0268142
	179 ^g	104.068	0.0806864
	254	1282.24	0.07899
	280 ^d	53.7885	0.0292034
	337	4749.31	0.0693429
I-5	151 ^f	19680.3	0.0752647
	192	510.634	0.00637564
	276 ^{b,de}	345021	0.00413544
I-6	234	73.7649	0.0575944
	321	868728	6.36E-05
	330	375.33	0.0012951
	337	756.867	0.0808631
Class I-Clade 2			
I-7	156	361.33	0.103665
	163 ^f	114.477	0.010233
	205	38.6138	0.0480599
	260 ^f	30.1684	0.100461
	287	266.611	0.0369899
I-8	105	48.9854	0.0968827
	156	112.156	0.0232119
	203	69.4737	0.090665
	210	193.948	0.0478296
	287	113.908	0.028478
	384	25.4691	0.0884657
I-9	105	14820	0.0520161
	253 ^g	15368.8	0.0141464
	291	232.766	0.0302471
	317 ^{cd}	21.1525	0.0549449
I-10	105	4963.49	0.0440919
	348	46.1381	0.0667295
I-11	187	22.3358	0.0845388
	195	784.304	0.100128

^aRelative position of amino acid sites in the class I chitinase multiple sequence alignment (A.5). ^bImportant for catalytic activity (Garcia-Casado et al. 1998). ^cResidue that putatively binds the substrate (Garcia-Casado et al. 1998). ^dPositioned in one of the seven active sites previously described for class I chitinases (Garcia-Casado et al. 1998; Bishop et al. 2000; Passarinho and de Vries 2002; Tiffen 2004). ^eResidue essential for substrate binding in the catalytic site but not for catalysis. Residue substitution at this site alters or eliminates catalytic activity (Verburg et al. 1992; Verburg et al. 1993). ^fPositionally homologous to site previously identified as under positive selection in *Arabidopsis* (Bishop et al. 2000). ^gPositionally homologous to site previously identified as under positive selection in *Zea* (Tiffen 2004).

Chapter Three

*Virus-induced gene silencing in the culinary ginger (*Zingiber officinale*): an effective mechanism for downregulating gene expression in tropical monocots*

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Abstract

Virus induced gene silencing (VIGS) has been shown to be effective for transient knockdown of gene expression in plants to analyze the effects of specific genes in development and stress related responses. VIGS is well established for studies model systems or crops within the Solanaceae, Brassicaceae, Leguminaceae and Poaceae, but only recently has been applied to plants residing outside these families. Here we have demonstrated that barley stripe mosaic virus (BSMV) can infect two species within the Zingiberaceae, and that BSMV-VIGS can be applied to specifically downregulate phytoene desaturase in the culinary ginger *Zingiber officinale*. These results suggest that extension of BSMV-VIGS to monocots other than cereals has the potential for directed genetic analyses of many important temperate and tropical crop species.

3.1 Introduction

Virus-induced gene silencing (VIGS) is a technique that utilizes the RNA interference (RNAi) pathway to down-regulate endogenous gene expression (Dinesh-Kumar et al. 2003; Burch-Smith et al. 2004; Godge et al. 2008). This process begins by abrading leaves with modified viral transcripts that express a plant cDNA sequence of a gene to be targeted for degradation (Kumagai et al. 1995; Ruiz et al. 1998). Once the transcripts begin replicating in vivo, double-stranded RNAs (dsRNAs) are generated by a viral RNA-dependent RNA polymerase, and the dsRNA intermediates are recognized by the plant's defense system and targeted for degradation into small interfering RNAs (siRNAs) by DICER-like enzymes (Benedito et al. 2004; Robertson 2004). Highly specific silencing of gene expression subsequently occurs as the amplified siRNAs are incorporated into RNA-induced silencing complexes (RISC) that degrade complementary endogenous plant mRNAs (Baulcombe 2004).

VIGS is a relatively new approach to down-regulate gene expression in plants. The technique was first applied with tobacco mosaic virus (TMV) to interfere with chlorophyll synthesis in *Nicotiana tabacum* L. (Kumagai et al. 1995). Later potato virus X (PVX-VIGS) was used to silence phytoene desaturase (PDS) in wild-type *Nicotiana benthamiana* Domin and to express green fluorescence protein (GFP) in transgenic *N. benthamiana* (Ruiz et al. 1998). However, tobacco rattle virus (TRV) has become the most widely used VIGS vector for members of the Solanaceae and Brassicaceae (Ratcliff et al. 2001; Burch-Smith et al. 2004; Chen et al. 2004; Fu et al. 2005; Burch-Smith et al. 2006; Dong et al. 2007; Godge et al. 2008), and the related pea early browning virus (PEBV) has been applied for developmental analysis of legumes (Constantin et al. 2004, 2008). TRV-VIGS has also recently been used for genetic analyses of the non-model basal eudicots, *Papaver somniferum* L. (Hileman et al. 2005; Drea et al. 2007), *Aquilegia* (Gould and Kramer 2007), and *Eschscholzia californica* Cham. (Wege et al. 2007). Among the cereal crops, VIGS using barley stripe mosaic virus (BSMV-VIGS) has been applied for barley (*Hordeum vulgare* L.) (Holzberg et al. 2002; Bruun-Rasmussen et al. 2007) and wheat (*Triticum aestivum* L.) (Scofield et al. 2005), but application of VIGS for monocots other than cereal grass species has not been described.

Because BSMV-VIGS has been very valuable for analysis of gene function in its natural host *Hordeum* (Hein et al. 2005; Oikawa et al. 2007; Shen et al. 2007) and in the closely related *Triticum* (Scofield et al. 2005; Cloutier et al. 2007; Fu et al. 2007; Zhou et al. 2007; Sindhu et al. 2008), we sought to determine whether the technology could be applied to tropical plants of the order Zingiberales. The Zingiberales (tropical ginger and bananas) exhibit a wide range of flower forms, making them an interesting system for investigating the role of specific gene families in the evolution of floral development (fig. 3.1). The order also exhibits substantial differences in growth habit; hence it is ideal for developmental studies on shoot, rhizome, and root systems. For this purpose, we designed a BSMV-VIGS vector to suppress PDS in the culinary ginger, *Zingiber officinale* Roscoe, using strategies similar to those successfully applied to barley (Holzberg et al. 2002) and wheat (Tai et al. 2005). Our results suggest wild-type (wt) BSMV is able to establish systemic infections of *Z. officinale* and *Costus spicatus* (Jacq.) Sw. We found that in *Z. officinale*, silencing of endogenous PDS (ZoPDS) results in white striations or fully photobleached leaves in systemically infected plants. We propose using *Z. officinale* as a model for studying gene function in non-grass monocots.

3.2 Materials and Methods

Testing infection with wildtype BSMV

Hordeum vulgare leaves infected for 5 to 6 days with the BSMV ND18 strain were ground in a mixture of 50 mL of 10 mM sodium phosphate buffer, pH 7.0 and 1% Celite Analytical Filter Aid (World Minerals). The extract was used to mechanically inoculate the leaves of young (~2 weeks after transplantation) vegetative shoots of *Z. officinale* (eight plants) and *C. spicatus* (four plants). Two leaves were inoculated per shoot and the plants were grown in ambient light under shading conditions in a whitewash-coated greenhouse maintained at 85% humidity. BSMV infection of the emerging leaves was indicated by visual observation (fig. 3.2B) and confirmed by RT-PCR of the RNA β subunit (fig. 3.3). At 14 days post infection (DPI), total RNA was extracted from ~0.5 g of leaf tissue using Purelink Plant Reagent protocol described by the supplier (Invitrogen) and the RNA was used for cDNA synthesis. For this purpose, 1.5 μ g of the total RNA extract primed with

the BSMV3 primer 5'-TGG-TCT-TCC-CTT-GGG-GGA-CCG-AAG-CT-3'. RT-PCR was performed using the forward primer TGB3 EcoRI 5'-GCG-AAT-TCC-ATG-GCA-ATG-CCT-CAT-CCC-C-3' and BSMV3 as the reverse primer with polymerase iTaq (BioRad Laboratories) and 5% DMSO at 95°C for 3 min, 35 cycles at 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 30 seconds and a single 7 min final extension cycle at 72°C.

PDS amplification

Total RNA was isolated from uninfected *Z. officinale* leaves with the Purelink Plant Reagent kit (Invitrogen) and subsequently used for cDNA synthesis using the iScript cDNA Synthesis Kit (BioRad Laboratories). To amplify PDS from the leaf RNA, the forward and reverse primers (5'-CTT-ATG-TTG-ARG-CYC-AAG-ATG-G-3' and 5'-GTG-TTC-TTS-AGT-TTT-CKR-TCA-AAC-3', respectively) were designed from a conserved region (fig. 3.4) in *Hydrilla verticillata*, *Lilium longiflorum*, *Crocus sativus*, *Zea mays*, *Oryza sativa*, *Hordeum vulgare* and *Triticum aestivum* (GenBank accession numbers AY639658, AY500378, AY183118, L39266, AF049356, AY062039 and DQ270236). A Polymerase Chain Reaction (PCR) was carried out utilizing the polymerase iProof kit (BioRad Laboratories) with 0.05 mg/mL BSA at 98°C for 4 min, 35 cycles at 98°C for 10 seconds, 62°C for 30°C seconds and 72°C for 30 seconds, and a single 7 min final extension cycle at 72°C.

Creating a ZoPDS VIGS construct

To apply BSMV-VIGS to *Z. officinale*, existing full-length cDNA plasmids derived from the ND18 strain (Petty et al. 1989) were used to generate RNAs for the infection mixture. These included the wild type RNA α plasmid and a modified BSMV β plasmid (B7), containing a mutation in the coat protein start codon (Petty and Jackson 1990). The B7 plasmid RNA was included in the infection mixture because Holzberg et al. (2002) have indicated that disruption of coat protein synthesis enhances the persistence of VIGS. The infection mixture also contained transcripts from the BSMV γ -ZoPDS plasmid, which is similar to the BSMV γ -TaPDS described for wheat VIGS by Tai et al. (2005). Both plasmids were derived from BSMV RNA γ - γ b*Bam*HI, which has an introduced *Bam*HI site that alters the start codon of the γ b ORF and blocks expression of the γ b protein (Petty 1990; Bragg and Jackson 2004). PDS cDNA amplified from *Z. officinale* was then digested with *Bam*HI and inserted non-directionally into the *Bam*HI site of BSMV RNA γ - γ b*Bam*HI to produce the BSMV γ -ZoPDS plasmid. Orientation of ZoPDS in the BSMV γ -ZoPDS transcript used for the infection mixture was determined to be positive via sequencing.

The BSMV plasmids were prepared separately for *in vitro* transcription reactions by linerization with Mlu1 (α and γ plasmids) or Spe1 (β plasmid), and synthesized *in vitro* in reactions containing ~500 ng of plasmid DNA and bacteriophage T7 RNA polymerase (Petty et al. 1989). After synthesis, the RNAs were extracted with phenol/chloroform, ethanol precipitated and resuspended in 50 μ l of 50 mM glycine, 30 mM sodium phosphate monobasic, 1% bentonite (Sigma) and 1% Celite (Petty et al. 1989). The RNAs were mixed, inoculated by rubbing transcripts directly onto two leaves of each plant, and the plants were grown as described above before leaf symptoms were evaluated at various times after inoculation. The BSMV γ -TaPDS construct containing the *T. aestivum* PDS gene was substituted for BSMV γ -ZoPDS in some experiments to

evaluate its effectiveness for VIGS in *Z. officinale*. The *T. aestivum* and *Z. officinale* PDS sequences have 77.3% sequence identity as determined by the program Geneious v3.7 (Drummond et al. 2007; available at <http://www.geneious.com/>). The nucleotide sequence for the ZoPDS gene was submitted to GenBank (accession number EU854153).

Quantifying PDS downregulation

Total RNA was isolated from uninfected control *Z. officinale* leaves, and leaves infected with BSMV γ -TaPDS and BSMV γ -ZoPDS. Tissue (~0.5 g) was ground in the presence of the Purelink Plant Reagent (Invitrogen) and extracted using the recommended procedures, and the extracts were subjected to DNase treatment (RQ1 RNase-Free DNase, Promega). The DNase-treated RNAs were subsequently used for total cDNA synthesis (iScript cDNA Synthesis Kit, BioRad Laboratories) and RT-PCR. PDS RNAs were amplified using the same primers as those used to amplify ZoPDS from cDNA, and the resulting products were analyzed by agarose gel electrophoresis. Forward and reverse primers for Actin (5'-GAT-GGA-TCC-TCC-AAT-CCA-GAC-ACT-GTA-3' and 5'-GTA-TTG-TGT-TGG-ACT-CTG-GTG-ATG-GTG-T-3', respectively) were used as controls during cDNA amplification with iProof polymerase (Biorad Laboratories) and 50 mg/ml of BSA.

3.3 Results

BSMV is able to infect members of the Zingiberales

BSMV has a very broad host range and infects several graminaceous hosts as well as some non-monocot species (Jackson and Lane 1981). Although there is a single report of *Commelina communis* L. (Commelinaceae; Commelinales) susceptibility (Jackson and Lane 1981), extensive studies have not been carried out on monocots belonging to families other than Poaceae, and, to the best of our knowledge, BSMV host range studies with the Zingiberales have not been conducted. Leaves of young *Z. officinale* shoots were inoculated with extracts of leaves from *H. vulgare* harboring the wt ND18 strain of BSMV. At 10 days after inoculation, newly emerging leaves developed a lightly striated mosaic phenotype (fig. 3.2B), and infection was confirmed with a Western blot for viral coat protein (CP) (fig. 3.3A) and by RT-PCR using primers targeting a 734-nt fragment within ORFs 3 and 4 of RNA β (fig. 3.3B). In addition to *Z. officinale*, we tested the susceptibility of the closely related *C. spicatus* to BSMV. We were able to confirm the presence of the BSMV in all inoculated plants by Western blotting (fig. 3.3A) and RT-PCR (fig. 3.3B) in all *C. spicatus*-inoculated individuals.

Interestingly, new shoots that developed from growing apices of rhizomes of plants previously infected with BSMV also developed symptoms of the viral infection. These shoots typically emerged 14 – 20 days post infection and do not appear to be delayed compared with uninfected plants. This observation supports past seed transmission and VIGS studies showing BSMV is able to infect meristematic tissue of grasses (Jackson and Lane 1981; Benedito et al. 2004). Our results also indicate that in *Z. officinale*, BSMV can move systemically from the primary inoculated leaves of a shoot into the rhizome system and into new shoots arising from the rhizome. Because of the growth habit of *Z. officinale*, in which many genetical identical shoots can be generated from the same

rhizome, only one shoot may need to be infected to obtain a large number of genotypically-identical infected plants bearing terminal flowering shoots.

BSMV can elicit VIGS of ZoPDS in ginger

To determine whether *Z. officinale* endogenous plant mRNAs can be silenced via a BSMV–VIGS approach, a fragment of the coding region of ZoPDS (GenBank accession number AF049356) was amplified by RT–PCR from *Z. officinale* mRNA. Once amplified, ZoPDS was sequenced and inserted at the 5' terminus of the γ b gene to create an infectious BSMV–VIGS vector unable to express the γ b protein (Tai et al. 2005). The ZoPDS fragment is an excellent gene for VIGS assays because it encodes for an enzyme involved in the biosynthesis of carotenoids and, once silenced, PDS is unable to protect chlorophyll from photo-oxidation, resulting in photobleaching due to decreased carotene content (Kumagai et al., 1995; Benedito et al., 2004). Silencing of PDS in *H. vulgare* (Holzberg et al. 2002) and *T. aestivum* (Tai et al. 2005) has been shown to reduce levels of carotene content and to result in an obvious photobleached phenotype.

Endogenous gene silencing by BSMV–VIGS was accomplished by inoculating leaves of eight young *Z. officinale* shoots through leaf abrasion with a combination of BSMV RNA transcripts designated BSMV γ –ZoPDS. This combination consisted of RNA α , a modified BSMV RNA β derivative (B7) that is deficient in expression of the coat protein (CP) (Petty and Jackson 1990), and BSMV RNA γ –ZoPDS transcripts. The RNA β and RNA γ modifications were introduced previously to enhance VIGS expression in barley and wheat (Holzberg et al. 2002; Tai et al. 2005). The 'B7' RNA β mutant was originally engineered to eliminate CP expression by mutagenesis of the AUG initiation codon of the CP ORF (Petty and Jackson 1990), and was used by Holzberg et al. (2002) to enhance BSMV–VIGS. Expression of the γ b silencing suppressor protein was also disrupted by creation of a *Bam*HI site to eliminate the γ b AUG (Petty et al. 1990) and to provide a site for insertion of cloned DNA fragments (Bragg and Jackson 2004).

Thirty days post inoculation with BSMV γ –ZoPDS, a silenced PDS photobleached phenotype appeared in the systemic leaves of all eight inoculated plants. Photobleaching was easily visible as partially or fully bleached sectors following the parallel venation along the length of the leaf blades (fig. 3.2C-D). Infected *Z. officinale* shoots developed varying degrees of photobleaching in new leaves and experienced slowed growth of the infected shoots, with high levels of mortality following complete bleaching of terminal leaves. Of eight plants inoculated that had only a single vegetative shoot, all lost their vegetative shoot apparently due to the death of the shoot apical meristem. Rhizomes remained viable, but did not display any signs of VIGS. wtBSMV-infected plants continued to grow and produce leaves and new shoots with only slight mosaic yellowing. In contrast to the bleaching with BSMV γ –ZoPDS, *Z. officinale* failed to develop a visible bleached phenotype after infection with BSMV γ –TaPDS (RNA α , B7 RNA β , and RNA γ –TaPDS) transcripts, which harbored *T. aestivum* PDS sequences.

ZoPDS transcripts are specifically downregulated in photobleached ginger

RT-PCR analyses revealed a dramatic reduction in the levels of PDS mRNA in the photobleached regions of the secondary leaves of plants inoculated with RNA γ -ZoPDS (fig. 3.4). As anticipated, RT-PCR analyses indicated that the secondary leaves of plants inoculated with BSMV γ -TaPDS had

levels of endogenous PDS mRNAs comparable to those of plants infected with the wild type BSMV ND18 strain or uninoculated plants (fig. 3.4). Further comparisons of *Triticum aestivum* and *Z. officinale* PDS sequences show a sequence identity of 77.3%, and varying degrees of identity are illustrated in other genera (fig. 3.5). A recent VIGS study in *Hordeum vulgare* shows that cDNA sequences used in viral vectors must have a high percentage of sequence identity to endogenous mRNA for VIGS to be successful (Fu et al. 2007). Our results indicate that BSMV-VIGS is just as sensitive to sequence identity in ginger as in grasses or in non-monocot systems (Burch-Smith et al. 2004; Godge et al. 2008; Scofield and Nelson 2009).

3.4 Discussion

The monocot order Zingiberales (tropical gingers) contains approximately 2500 species that form specialized pollination relationships via alterations in floral form. Members of the order comprise a major component of both tropical and subtropical ecosystems and include crop plants (e.g. banana, plantain, ginger), sources of traditional medicines and spices (e.g. cardamom, turmeric, galanga), and horticulturally important ornamentals (e.g. Heliconia, Bird-of-Paradise, Canna). Detailed studies of two families, Costaceae and Zingiberaceae, indicate that specialized relationships with animal pollinators have led to increased rates of diversification (i.e. rapid radiations) in bird-pollinated and bee-pollinated lineages (Specht 2005, 2006). Species within these two families thus represent ideal evolutionary models for comparative morphology and developmental genetic studies.

Of the tropical gingers, *Zingiber officinale* is the most extensively described and widely cultivated for use of its aromatic rhizomes in cooking and home remedies. Extracts from *Z. officinale* have been shown to have pharmacological activities, and may be effective in inhibiting a variety of illnesses, including the promotion of tumors, inflammation, and emesis (Kawai et al. 1994; Katiyar et al. 1996; Penna et al. 2003). *Zingiber officinale* and related species are easily cultivated vegetatively from rhizome cuttings or vegetative bulbils. This characteristic obviates the need for seed production for successful reproduction and permits rapid generation of multiple genetically identical individuals. In addition, gingers are herbaceous perennials with fast growth rates and short times to maturity (6–7 months seed to seed), making them realistically amenable to gene silencing and subsequent phenotyping. In order to further understand and add to research of the Zingiberales, we demonstrate that virus-induced gene-silencing (VIGS) using a cereal virus, barley stripe mosaic virus (BSMV), can be used effectively to study gene function in *Z. officinale*.

BSMV infection of Zingiberales

While *Hordeum vulgare* (subfamily Pooideae: tribe Triticeae) is the natural host for BSMV, systemic viral infection with BSMV has been demonstrated for most subfamilies and many tribes of grasses (Jackson and Lane 1981). BSMV-VIGS has been successfully demonstrated for barley and wheat (Pooideae: Triticeae) and is proving to be extremely valuable for analysis of genes affecting morphogenesis and disease resistance (Hein et al. 2005). Here, we demonstrate that BSMV systemic infections of distantly-related monocots, such as gingers, occurs after mechanical inoculation with both wild type and engineered ND18 strain transcripts. Moreover, infections can be maintained in *Zingiber officinale* (Zingiberaceae) and a closely related species in the family Costaceae (*Costus spicatus*) for at least 5 months during normal greenhouse growing conditions.

Plants infected with BSMV ND18 developed a mild yellow mosaic on the normally bright green leaves, but did not show obvious reductions in overall plant growth or in the timing of transition from vegetative to reproductive phase. In the absence of intentional leaf abrasion, BSMV does not appear to be easily transmitted to surrounding plants in the greenhouse. This is an important practical feature for gene silencing in the Zingiberales since many plants become too large for growth in chambers and require high levels of humidity that are difficult to maintain if plants are grown in isolation.

In each of the tested species, BSMV moved from the initial sites of infection to developing leaves above the site of infection. Additionally, in *Zingiber* we observed the movement of the virus downward through the infected shoot and into the rhizome (underground stem), where it ultimately infected new shoots developing from the rhizome tip (see fig. 3.1). The recently reported stability of BSMV VIGS (Bruun-Rasmussen et al. 2007) demonstrates the potential to control gene expression for a considerable period of time during plant development. In *Z. officinale* BSMV persists over several vegetative and flowering cycles, and presents the opportunity to create a large number of ginger shoots with down-regulated gene expression from inoculation of a single leaf. After a cluster is infected, we should be able to grow individual shoots separately to test the effects of gene down-regulation under a variety of environmental conditions.

BSMV-VIGS is effective in *Zingiber officinale*

We have shown that BSMV-VIGS is an efficient method for inducing the down-regulation of the expression of specific target genes in *Z. officinale*. A modified BSMV γ RNA containing a partial sequence of the *Z. officinale* phytoene desaturase (PDS) gene was able to effect the down-regulation of endogenous PDS and cause visible photo bleaching of leaf and stem tissue. The extent of PDS silencing in *Z. officinale* after BSMV-VIGS inoculation is similar to that found in studies of PDS VIGS in other monocots (Holzberg et al. 2002; Tai et al. 2005). However, *Z. officinale* shoots with photobleached leaves showed high rates of mortality, and rhizomes were not able to produce new shoots or develop inflorescences for over 50 days after inoculation. The vegetative shoots showing PDS eventually died, and failed to regenerate photosynthetic tissue, suggesting complete gene silencing in the shoot apical meristem. It is therefore likely that gene silencing will be an effective means to elucidate the functions of genes involved in developmental and biochemical pathways. Future analyses with other marker genes that do not lead to photo-oxidation are planned to determine the duration of gene down-regulation as well as to test the physical movement of gene silencing throughout the plant.

VIGS has become a widely used technique, most commonly applied to eudicot plants using tobacco rattle virus (TRV) derived vectors and *Agrobacterium*-mediated transfer into host cells. Despite reports of a host range for TRV that includes monocots (see TEC Release October 2005: www.pbltechnology.com), we were unable to infect Zingiberales using *Agrobacterium*-mediated infiltration of TRV in several attempts using various published delivery methods. The use of a native monocot virus to affect VIGS in phylogenetically distant monocot taxa presents an effective means of transferring VIGS technology to a wide range of crop species, model organisms, and non-model species within the monocots. This transfer of technology provides a high-throughput means for assaying the function of a large number of genes recently identified and sequenced through EST databases and genome sequencing projects being developed for a number of diverse grasses (*Zea mays*, *Oryza sativa*, *Hordeum vulgare*, *Triticum* spp., *Sorghum* spp., *Panicum virgatum*, *Brachypodium*

distachyon, *Saccharum officinarum*) and non-grass monocots (*Musa acuminata*, *Asparagus officinalis*, *Phalaenopsis* spp., *Ananas comosus*, *Allium cepa*). The simple topical application method for introduction of the virus increases the ease at which BSMV can be used to assess gene function across monocots. We are currently testing the efficacy of the virus in infecting species of *Allium* (Alliaceae; Asparagales), *Hippeastrum* (Amaryllidaceae; Asparagales), *Iris* (Iridaceae; Asparagales), *Acorus* (Acoraceae; Acorales), and *Chamaedorea* (Arecaceae; Arecales).

In addition to studying host gene function, VIGS has the potential to provide a useful method for assaying host factors involved in viral pathogenicity (Zhu and Dinesh-Kumar 2008). Biochemical assays have been used to identify various host translation initiation factors associated with viral replication proteins (Quadt et al. 1993). VIGS provides an additional means for testing the function of candidate host factors in viral pathogenicity, providing a high-throughput mechanism for screening potential new host factors and testing for the effects of candidate host factors on pathogenesis. The recent spread of the vector-borne banana virus, Banana bunchy top virus (BBTV), has resulted in the spread of banana bunchy top disease and the subsequent failure of banana crops in Hawaii (Conant 1992) and across the South Pacific and Southeast Asia (Dale 1987). BSMV-VIGS in banana (*Musa acuminata*; Musaceae; Zingiberales) could provide a reverse genetics approach to help elucidate host mechanisms involved in viral pathogenicity.

BSMV-VIGS as a tool for studying gene function in the Zingiberales

BSMV-VIGS is likely to be effective in other members of the Zingiberales that are susceptible to BSMV infection. This should enable targeted studies for identifying gene function to be carried out in this ecologically and evolutionarily important group of tropical crops and ornamentals. The ginger family, Zingiberaceae, includes species such as turmeric (*Curcuma longa* L.), galanga (*Kaempferia galanga* L.), cardamom (*Elettaria cardamomum* Maton), and ginger root (*Z. officinale*), all of which have uses as spices and medicinals. Most rhizomes of Zingiberaceae species accumulate high levels of pharmacologically active metabolites derived from the phenylpropanoid pathway. Several of these, gingerols in *Zingiber* and curcuminoids in *Curcuma*, have been isolated and characterized, but little is known about their biosynthesis. Recent biochemical studies have started to identify enzymes involved in the biosynthetic pathways (Ramirez-Ahumada et al. 2006; Kita et al. 2008); however, nothing is known about the genetic network involved in biosynthesis. Our developed BSMV-VIGS tool could be used to functionally analyze ESTs believed to be associated with the biosynthesis of these important compounds.

Our interest in developing BSMV-VIGS in Zingiberales extends to floral developmental evolution. We are interested in dissecting the genetic networks leading to development of the diverse floral forms found across the order, particularly floral forms involved in the attraction of distinct pollinators. Unlike grasses, Zingiberales are 'petaloid monocots', having floral organs comprising sepals, petals, stamens, and carpels. The formation of the staminodes and the labellum may be a question of organ identity, with these structures functionally homologous to petals yet sharing positional homology with stamens. A group of transcription factors, many of which belong to the MADS-box family, are involved in floral organ identity in several model plant systems (Saedler et al. 2001; Theissen 2001). VIGS has been successfully used to study MADS-box gene function in a variety of eudicots (Schwartz-Sommer et al. 1990; Liu et al. 2004; Hileman et al. 2005; Drea et al. 2007; Gould and Kramer 2007) and future studies using VIGS may allow us to determine how these organ identity genes influence floral form throughout the Zingiberales (e.g. Gao et al. 2006).

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Fig. 3.1 Photos of the two species used in this study, clockwise from upper left. (A). Inflorescence of *Costus spicatus*, showing current and past flowers. One flower opens per day from the conical inflorescence. (B). Close-up of *C. spicatus* flower (credit: Madelaine E. Bartlett). (C). *Zingiber officinale* inflorescence with flowers. (D,E). *Zingiber officinale* shoots and rhizomes showing facility for replication of greenhouse experiments. (D) Close-up of the three rhizome sections shown in E. The growing end of the rhizome is toward the bottom of the photograph, seen as a small protrusion of the rhizome tissue. This protrusion represents a single apical meristem and will grow out as a new vegetative shoot. Lateral thickening at the base will form the rhizome, and roots will develop from axillary meristems along the rhizome. (E). Three rhizome sections with corresponding shoot and root systems, demonstrating 4 months of growth. Each rhizome section can be separated from adjoining sections and planted as an individual, complete with shoot and root system. Flowering, leafless shoots (C) form directly off the rhizome.

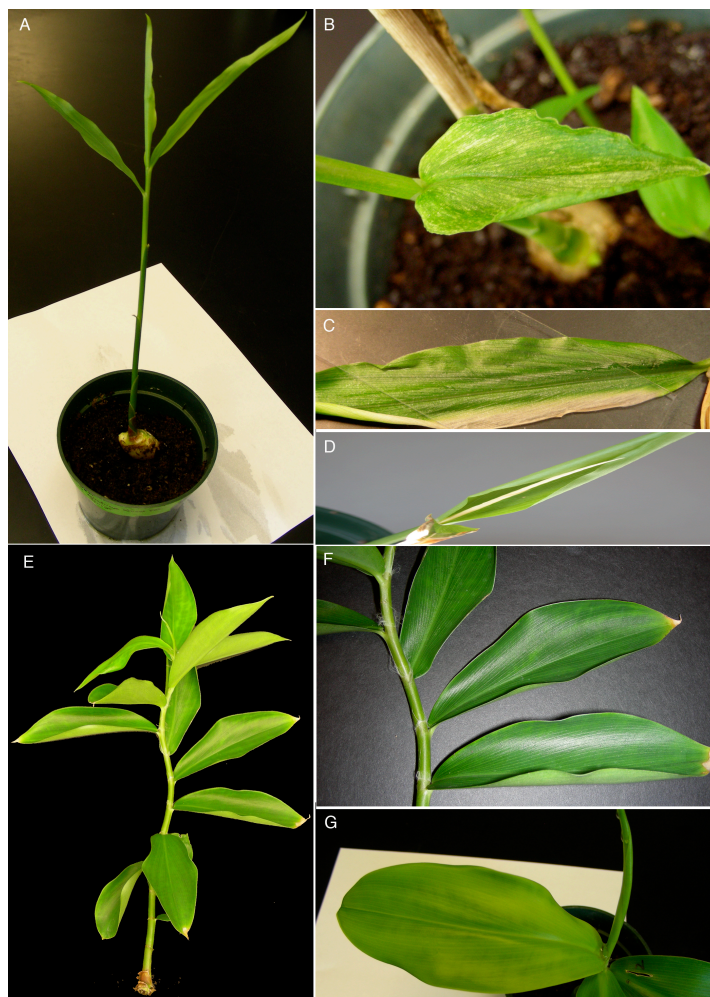


Fig. 3.2 Images of *Zingiber officinale* (A-D) and *Costus spicatus* (E-G) demonstrating phenotypes associated with viral infection and gene silencing. (A) Whole *Z. officinale* plant with rhizome. (B) Leaf from BSMV infected *Z. officinale* plant showing characteristic yellow stripes. (C) Leaf from a *Z. officinale* plant systemically infected with BSMV-ZoPDS. Photobleaching is evident in one sector of the single leaf located directly above the leaf of infection and can be seen throughout the terminal leaves of plants 30 days post-infection. (D) Terminal leaf of *Z. officinale* plant with complete photobleaching characteristic of PDS silencing. (E) Whole *C. spicatus* stem produced from bulbil in the leaf axil of the parent flowering plant. The rhizome at the base of the shoot has rootlets that developed while this stem was still attached to the parent plant. (F) Close-up of uninfected *C. spicatus* leaves. (G) Close-up of *C. spicatus* leaf infected with BSMV, showing the infected phenotype of characteristic yellow stripes in a mosaic pattern.

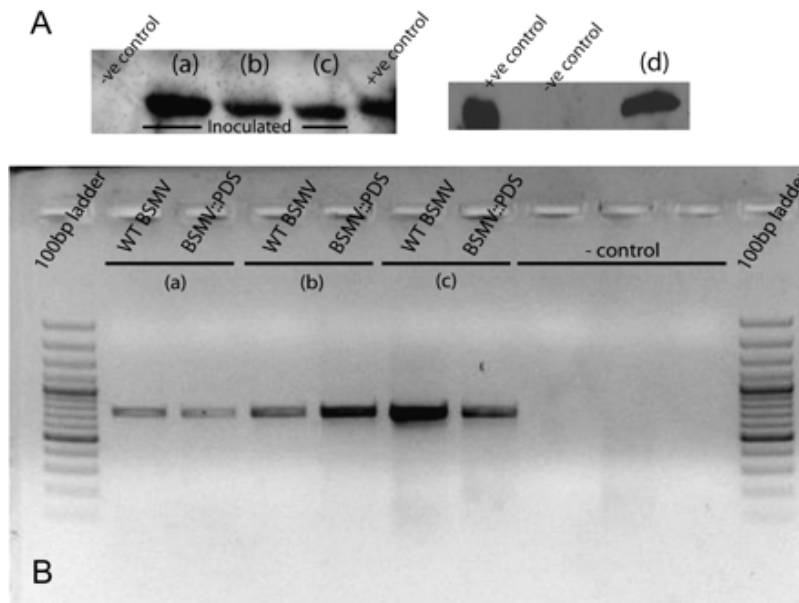


Fig. 3.3 (A) Western blots showing antibody hybridization to BSMV CP. Detected CP in inoculated lanes indicates viral replication >3 cm from site of abrasion in 3 different *Costus* plants (a-c) and in *Z. officinale* (d). Negative control lanes use leaf material harvested from uninfected *C. spicatus* (left blot) or *Z. officinale* (right blot). Positive control lanes on both blots use leaf material from barley (*Hordeum*) known to be systemically infected with BSMV. (B) RT-PCR showing presence of viral constructs in plants post-inoculation with either wt BSMV or BSMV RNA with the PDS insert. Primers were designed to amplify BSMV β RNA. Control lanes contain RT-PCR from plants not infected with viral transcripts. (a) *C. spicatus*, (b) *Z. officinale*, (c) *H. vulgare*.

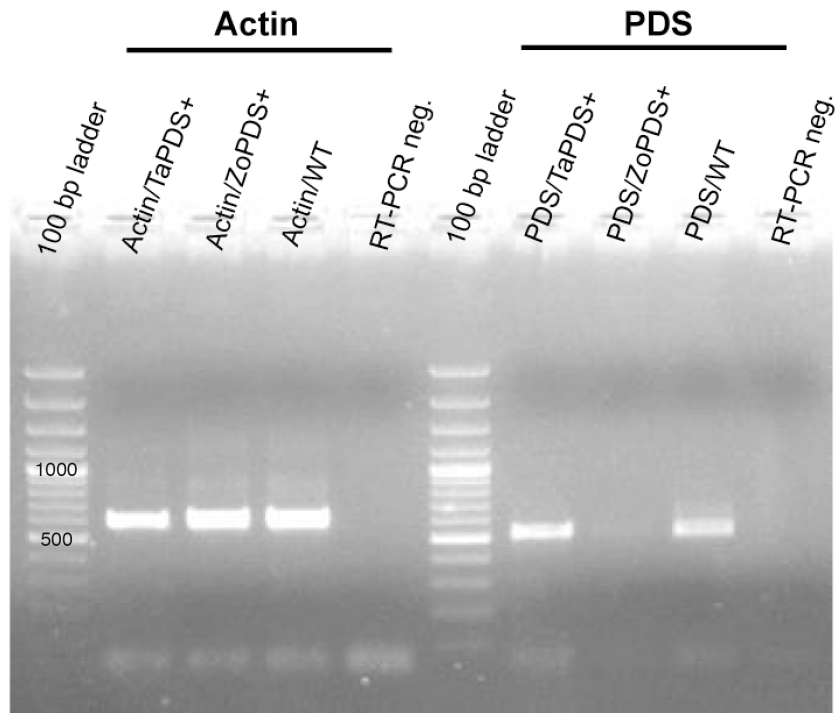


Fig. 3.4 Agarose gel with RT-PCR demonstrating down-regulation of PDS in *Z. officinale* plants infected with a BSMV construct containing a *Z. officinale* endogenous PDS gene fragment. Actin was used as a positive RT-PCR control for RNA extractions from *Z. officinale* plants infected with BSMV containing no construct (WT), a fragment of the PDS gene from wheat (Ta), or the endogenous *Z. officinale* PDS fragment (Zo). In all cases, actin is consistently transcribed. Significantly less PDS is transcribed from plants infected with the BSMV vector containing the endogenous PDS gene fragment, indicating successful downregulation of *PDS* (ZoPDS+). The negative control reaction lacks reverse transcriptase.

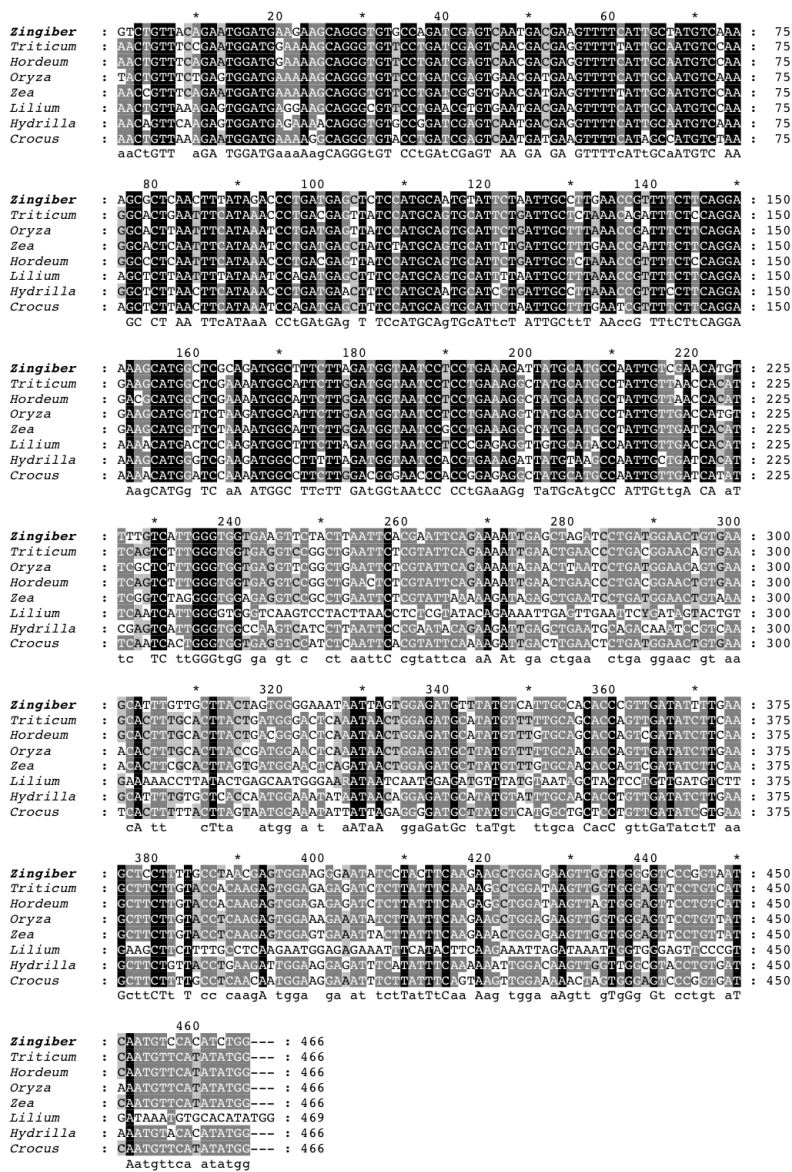


Fig. 3.5 Sequence comparison of PDS loci from *Zingiber officinale*, *Triticum aestivum*, *Hordeum vulgare*, *Oryza sativa*, *Zea mays*, *Crocus sativus*, *Lilium longiflorum*, *Hydrilla verticillata* (GenBank accession numbers EU854153, DQ270236, AF049356, AY062039, L39266, AY183118, AY500378, AY639658) utilizing the program GeneDoc version 2.06.02 (Nicholas et al. 1997). Nucleotides shaded by black boxes with white lettering (100% conserved), gray shading with white lettering (80% or greater conserved), gray shading with black lettering (60% or greater conserved), no shading with black lettering (less than 60% conserved).

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Appendices

A.1

Voucher information for collected specimens utilized in chapter 1

The voucher information includes the names of taxa used in this study, details of the tissue collection, herbaria where the vouchers are deposited, and GenBank accession numbers for the sequences of ITS, PY-IGS, *atpB*, *matK*, *petB*, PTR1, *rbcL*, and *matK*. Greenhouse-grown specimens cultivated at the Botanical Gardens of the University of California, Berkeley, California Carnivores, Missouri Botanical Garden or elsewhere are noted after the voucher information. California Carnivores, Sebastopol, CA (Peter D'Amato) = CC, Indonesian Institute of Sciences/Center for Plant Conservation-Bogor Botanical Gardens = LIPI/CPCBG, Missouri Botanical Garden = MO, Botanical Garden of the University of California, Berkeley = UCBG, Botanischer Garten der Universität Würzburg = BGW. Voucher specimens are deposited in the following herbaria: Missouri Botanical Garden = MO, The University and Jepson Herbaria of the University of California, Berkeley = UC, Universität Würzburg = UW. Dashes indicate missing data.

Taxon; ITS, PY-IGS, *atpB*, *matK*, *petB*, PTR1, *rbcL*, *matK*; Voucher specimen or living collection number; DNA collection number; Collection locale; Herbarium.

Aldrovanda vesiculosa; HM204865, HM204823, AY096108, AY096120, –, –, AY096106, –, –, TR174; USA, New Jersey, cultivated (R. Sivertsen); UC.

Ancistrocladus abbreviatus; HM204866, –, –, AF204840, FN598602, –, –, AF315939; 97-12-B-10; TR114; BGW, cultivated; UW. *Ancistrocladus barteri*; HM204867, HM204824, –, –, –, –, –, –, 05-87-B-10; TR118; BGW, cultivated; UW. *Ancistrocladus benomensis*; HM204868, HM204825, –, –, –, –, –, –, 00-54-B-20; TR117; BGW, cultivated; UW. *Ancistrocladus cochinchinensis*; HM204869, HM204826, –, –, –, –, –, –, 04-96-B-20; TR120; BGW, cultivated; UW. *Ancistrocladus congolensis*; HM204870, –, –, –, –, –, –, –, 00-29-B-10; TR119; BGW, cultivated; UW. *Ancistrocladus grandiflorus*; HM204871, HM204827, –, –, –, –, –, –, R.E. Gereau 5557; TR122; MO, cultivated; MO. *Ancistrocladus guineensis*; HM204872, HM204828, –, –, –, –, –, –, R.E. Gereau 5546; TR125; MO, cultivated; MO. *Ancistrocladus hamatus*; HM204873, HM204829, –, –, –, –, –, –, TR113; LIPI/CPCBG, cultivated; –. *Ancistrocladus heyneanus*; HM204874, HM204830, –, AF204841, –, –, –, GQ470529; 95-49-B-10; TR115, BGW, cultivated; UW. *Ancistrocladus korupensis*; HM204875, HM204831, AF209526, AF204839, –, –, Z97636, GQ470536; 99-3-B-10; TR116; BGW, cultivated; UW. *Ancistrocladus letestui*; –, HM204832, –, –, –, –, –, –, R.E. Gereau 5566; TR123; MO, cultivated; MO. *Ancistrocladus robertsoniorum*; HM204876, HM204833 –, –, –, –, –, –, K.M. Meyer 277; TR124; MO, cultivated; MO.

Dionaea muscipula; HM204877, HM204834, AY096112, –, FN598597, –, DONCPRBCL, –, 2009.0254; TR186; UCBG, cultivated; UC.

Dioncophyllum tholloni; HM204878, HM204835, –, AF204844, –, –, –, –, G. Walters 1948; TR187; Gabon, Haut-Ogooué, Batéké Plateaux; MO.

Drosera binata; HM204879, HM204836, –, –, –, –, DRSCPRBCL, –, 2001.0104; TR03; UCBG, cultivated; UC. *Drosera capensis*; HM204880, –, –, AY096122, –, –, DRSCPRBCB, –, 69.0172; TR05; UCBG, cultivated; UC. *Drosera dielsiana*; HM204881, –, –, –, –, –, –, TR0004;

TR41; CC, cultivated; UC. *Drosera falconeri*; HM204882, -, -, -, -, -, -, -; TR0073; TR112; CC, cultivated; UC. *Drosera graminifolia*; HM204883, -, -, -, -, -, -, -; TR0015; TR53; CC, cultivated; UC. *Drosera hamiltonii*; HM204884, -, -, -, -, -, -, -; TR0009; TR47; CC, cultivated; UC. *Drosera nidiformis*; HM204885, -, -, -, -, -, -, -; TR0003; TR40; CC, cultivated; UC. *Drosera paleacea*; HM204886, -, -, -, -, -, -, -; TR0011; TR49; CC, cultivated; UC. *Drosera regia*; HM204887, HM204837, AY096111, AF204848, FN598596, -, DRSCPRBCG, -; TR0002; TR39; CC, cultivated; UC. *Drosera rotundifolia*; HM204888; HM204838, -, -, AB298084, -, AB072538, -; TR0024; TR62; CC, cultivated; UC. *Drosera slackii*; HM204889, -, -, -, -, -, -, -; TR0001; TR38; CC, cultivated; UC.

Drosophyllum lusitanicum; HM204890; HM204839, AY096113, AF204846, FN598600, -, DRHCPRBCLA, AY514860; TR0023; TR61; CC, cultivated; -.

Limonium; EU410356, -, AF209620, AY042610, FN598585, -, AF206789, AY514861; -; -; -.

Nepenthes alata; HM204891, HM204840, AF093388, AF204834, -, AF080545, NETCPRBCL, AF315891; 87.0830; TR09; UCBG, cultivated; UC. *Nepenthes albomarginata*; HM204892, HM204841, -, -, -, -, -, -; 95.1376; TR10; UCBG, cultivated; UC. *Nepenthes boschiana*; HM204893, HM204842, -, -, -, -, -, -; 2004.0625; TR11; UCBG, cultivated; UC. *Nepenthes glandulifera*; HM204895, HM204844, -, -, -, -, -, -; 2005.1319; TR14; UCBG, cultivated; UC. *Nepenthes gracillima*; HM204896, HM204845, -, -, -, -, -, -; 95.1453; TR15; UCBG, cultivated; UC. *Nepenthes gymnamphora*; HM204897, HM204846, -, -, -, -, -, -; 95.1499; TR16; UCBG, cultivated; UC. *Nepenthes hirsuta*; -, HM204847, -, -, -, -, -, -; 93.0479; TR17; UCBG, cultivated; -. *Nepenthes insignis*; HM204898, HM204848, -, -, -, -, -, -; 95.1399; TR19; UCBG, cultivated; UC. *Nepenthes macfarlanei*; HM204900, HM204850, -, -, -, -, -, -; 95.1300; TR21; UCBG, cultivated; -. *Nepenthes maxima*; HM204901, HM204851, -, -, -, -, -, -; 76.1342; TR22; UCBG, cultivated; UC. *Nepenthes mirabilis*; HM204902, HM204852, -, -, -, -, -, -; 95.1378; TR23; UCBG, cultivated; UC. *Nepenthes northiana*; HM204903, HM204853, -, -, -, -, -, -; 95.1124; TR24; UCBG, cultivated; UC. *Nepenthes rafflesiana*; HM204904, HM204854, -, -, -, -, -, -; 69.0037; TR25; UCBG, cultivated; UC. *Nepenthes reinwardtiana*; HM204905, HM204855, -, -, -, -, -, -; 87.0822; TR26; UCBG, cultivated; -. *Nepenthes sanguinea*; HM204906, HM204856, -, -, -, -, -, -; 95.1297; TR27; UCBG, cultivated; UC. *Nepenthes singalana*; HM204907, HM204857, -, -, -, -, -, -; 95.1292; TR29; UCBG, cultivated; UC. *Nepenthes spectabilis*; HM204908, HM204858, -, -, -, -, -, -; 95.1516; TR31; UCBG, cultivated; UC. *Nepenthes tentaculata*; HM204909, HM204859, -, -, -, -, -, -; 93.0483; TR32; UCBG, cultivated; UC. *Nepenthes tobiaca*; -, HM204860, -, AF204829, -, DQ840233, -, AF315899; 95.1501; TR33; UCBG, cultivated; UC. *Nepenthes truncata*; HM204910, HM204861, -, -, -, -, -, -; 87.0829; TR34; UCBG, cultivated; UC. *Nepenthes ventricosa*; HM204911, HM204862, -, AF204833, -, -, FJ860397, AF315892; 91.1046; TR35; UCBG, cultivated; UC. *Nepenthes vieillardii*; HM204912, HM204863, -, -, -, AB232943, AB103319, AF315897; 95.1349; TR37; UCBG, cultivated; UC.

Polygonum; GQ206260, EU840331, AJ235569, EF438020, FN598591, -, AF297127, EF653710; -; -; -.

Triphyophyllum peltatum; HM204913, HM204864, -, AF204843, FN598601, -, -, AF315940; UW plant culture; TR121; BGW, cultivated; -.

A.2

Multiple sequence alignment of combined nDNA, nrDNA, and cpDNA molecular marker phylogenetic reconstruction for the carnivorous Caryophyllales

The following 21 pages consist of a multiple sequence alignment of combined nDNA, nrDNA, and cpDNA molecular marker phylogenetic reconstruction for the carnivorous Caryophyllales. ITS = 1 - 1705; PYIGS = 1706 - 3075; *atpB* = 3076 - 4583; *petB* = 4584 - 5721; *matK* = 5722 - 7185; PTR1 = 7186 - 8975; *rbcL* = 8976 - 11142; *trnK* = 11143 - 13773. GAP = - and MISSING = ? The following sites were excluded for analyses: 1 - 32, 55 - 79, 91 - 608, 629 - 909, 1082 - 1191, 1217 - 1258, 1290 - 1353, 1376 - 1639, 1671 - 1705, 1925 - 1960, 2035 - 2199, 2355 - 2889, 3076 - 3134, 4530 - 4583, 4584 - 4601, 5706 - 5721, 5722 - 6035, 7181 - 7185, 7186 - 7306, 8892 - 8975, 8976 - 9774, 11081 - 11142, 11143 - 11150, 11779 - 11835, 12196 - 12217, 13487 - 13518, 13535 - 13553, 13766 - 13773.

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Ancistrocladus_abbreviatus
Ancistrocladus_heyneanus
Ancistrocladus_robertysoniorum
Dionaea_muscipula
Drosera_binata
Drosera_regia
Drosera_rotundifolia
Limonium
Nepenthes_alata
Nepenthes_tobaiaca
Nepenthes_venitricosa
Nepenthes_vieillardii
Polygonum
Triphyophyllum_peltatum
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Aldrovanda_vesiculosa	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Ancistrocladus_abbreviatus	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Ancistrocladus_leyreanus	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Ancistrocladus_robertysoniorum	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Dionaea_muscipula	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Dioscophyllum_thollonii	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Drosera_binata	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Drosera_regia	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Drosera_rotundifolia	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Limnium	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Nepenthes_alata	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Nepenthes_burkeana	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Nepenthes_ventricosa	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Nepenthes_vieillardii	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Polyponum	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Triphyophyllum_peltatum	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Aldrovanda_vesiculosa	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Ancistrocladus_abbreviatus	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Ancistrocladus_leyreanus	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Ancistrocladus_robertysoniorum	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Dionaea_muscipula	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Dioscophyllum_thollonii	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Drosera_binata	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Drosera_regia	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Drosera_rotundifolia	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Limnium	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Nepenthes_alata	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Nepenthes_burkeana	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Nepenthes_ventricosa	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Nepenthes_vieillardii	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Polyponum	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770
Triphyophyllum_peltatum	13,690	13,700	13,710	13,720	13,730	13,740	13,750	13,760	13,770

A.3

Multiple sequence alignment of ITS and PY-IGS phylogenetic reconstruction for the carnivorous Caryophyllales

The following 37 pages consist of a multiple sequence alignment of ITS and PY-IGS phylogenetic reconstruction for the carnivorous Caryophyllales. ITS = 1 – 1705 and PYIGS = 1706 – 3075. GAP = - and MISSING = ? The following sites were excluded for analyses: 1 – 32, 55 – 79, 91 – 608, 629 – 909, 1082 – 1191, 1217 – 1258, 1290 – 1353, 1376 – 1639, 1671 – 1705, 1925 – 1960, 2035 – 2199, 2355 – 2889.

	1	10	20	30	40	50	60	70	80
Aldrovanda vesiculosa	?	?	?	?	?	?	?	?	?
Ancistrocladus abbreviatus	?	?	?	?	?	?	?	?	?
Ancistrocladus_barteri	?	?	?	?	?	?	?	?	?
Ancistrocladus_benomensis	?	?	?	?	?	?	?	?	?
Ancistrocladus_cochichinensis	?	?	?	?	?	?	?	?	?
Ancistrocladus_congolensis	?	?	?	?	?	?	?	?	?
Ancistrocladus_grandiflorus	?	?	?	?	?	?	?	?	?
Ancistrocladus_guineensis	?	?	?	?	?	?	?	?	?
Ancistrocladus_hamatus	?	?	?	?	?	?	?	?	?
Ancistrocladus_heyneanus	?	?	?	?	?	?	?	?	?
Ancistrocladus_korupensis	?	?	?	?	?	?	?	?	?
Ancistrocladus_letestui	?	?	?	?	?	?	?	?	?
Ancistrocladus_robertsoniorum	?	?	?	?	?	?	?	?	?
Dionaea_muscipula	?	?	?	?	?	?	?	?	?
Dionophyllum_thollonii	?	?	?	?	?	?	?	?	?
Drosera_binata	?	?	?	?	?	?	?	?	?
Drosera_capensis	?	?	?	?	?	?	?	?	?
Drosera_dielisiana	?	?	?	?	?	?	?	?	?
Drosera_falconeri	?	?	?	?	?	?	?	?	?
Drosera_graminifolia	?	?	?	?	?	?	?	?	?
Drosera_nidiformis	?	?	?	?	?	?	?	?	?
Drosera_paleacea	?	?	?	?	?	?	?	?	?
Drosera_regia	?	?	?	?	?	?	?	?	?
Drosera_rotundifolia	?	?	?	?	?	?	?	?	?
Drosera_slackii	?	?	?	?	?	?	?	?	?
Drosophyllum_lusitanicum	?	?	?	?	?	?	?	?	?
Nepenthes_alata	?	?	?	?	?	?	?	?	?
Nepenthes_albomarginata	?	?	?	?	?	?	?	?	?
Nepenthes_boschiana	?	?	?	?	?	?	?	?	?
Nepenthes_fusca	?	?	?	?	?	?	?	?	?
Nepenthes_glandulifera	?	?	?	?	?	?	?	?	?
Nepenthes_gracillima	?	?	?	?	?	?	?	?	?
Nepenthes_gymnamphora	?	?	?	?	?	?	?	?	?
Nepenthes_hirsuta	?	?	?	?	?	?	?	?	?
Nepenthes_ignis	?	?	?	?	?	?	?	?	?
Nepenthes_macfarlanei	?	?	?	?	?	?	?	?	?
Nepenthes_maxima	?	?	?	?	?	?	?	?	?
Nepenthes_mirabilis	?	?	?	?	?	?	?	?	?
Nepenthes_northiana	?	?	?	?	?	?	?	?	?
Nepenthes_rafflesiana	?	?	?	?	?	?	?	?	?
Nepenthes_reinwardtiana	?	?	?	?	?	?	?	?	?
Nepenthes_sanguinea	?	?	?	?	?	?	?	?	?
Nepenthes_singalana	?	?	?	?	?	?	?	?	?
Nepenthes_spectabilis	?	?	?	?	?	?	?	?	?
Nepenthes_tentaculata	?	?	?	?	?	?	?	?	?
Nepenthes_tobiaca	?	?	?	?	?	?	?	?	?
Nepenthes_truncata	?	?	?	?	?	?	?	?	?
Nepenthes_ventricosa	?	?	?	?	?	?	?	?	?
Nepenthes_vieillardii	?	?	?	?	?	?	?	?	?
Triphyophyllum_peltatum	?	?	?	?	?	?	?	?	?

Aldrovanda_vesiculosa	90	100	110	120	130	140	150	160
Ancistrocladus_abbreviatus	???	???	???	???	???	???	???	???
Ancistrocladus_barteri	???	???	???	???	???	???	???	???
Ancistrocladus_benomensis	CATGTT	---	---	---	---	---	---	---
Ancistrocladus_cochichinensis	CATGTT	---	---	---	---	---	---	---
Ancistrocladus_congolensis	CATGTT	---	---	---	---	---	---	---
Ancistrocladus_grandiflorus	CATGTT	---	---	---	---	---	---	---
Ancistrocladus_guineensis	CATGTT	---	---	---	---	---	---	---
Ancistrocladus_hamatus	CATGTT	---	---	---	---	---	---	---
Ancistrocladus_heyneanus	CATGTT	---	---	---	---	---	---	---
Ancistrocladus_korupensis	???	???	???	???	???	???	???	???
Ancistrocladus_letestui	???	???	???	???	???	???	???	???
Ancistrocladus_robertsoniorum	???	???	???	???	???	???	???	???
Dionaea_muscipula	???	???	???	???	???	???	???	???
Dioncophyllum_thollonii	CTTGT	AAACT	AAAAT	GAAAGG	-GGGAAG	---	-CCTTCT	CCCCC
Drosera_binata	CGTGT	CAACC	-AAAT	AGAGG	-GATGGG	CGTGT	CGTGT	CGCC
Drosera_capensis	???	???	???	???	???	???	???	???
Drosera_dielisiana	???	???	???	???	???	???	???	???
Drosera_falconeri	CGAGT	TAAAG	-TAAC	TAAACGG	-GGG	-GG	TATGA	TGTGT
Drosera_rotundifolia	GGAGG	GCG	-TGGCT	CGGA	AG	---	-CFTTGC	---
Drosera_graminifolia	CGTGT	CAACC	-AAAC	AGAGG	-GA	-CCGAG	CGCG	CGTGC
Drosera_hamiltonii	CGTGT	CAACC	-AAAC	AGAGG	-GACGG	-GG	CGCGT	CGTGC
Drosera_nidiformis	CGTGT	CAACC	-AAAT	AGAGG	-GATGG	-GG	TGTGT	CGTGC
Drosera_paleacea	CATGT	TAACT	AAAAT	ATGGG	TGGGGGTG	---	-TCTTTT	---
Drosera_regia	CGTGT	CAACC	-AAT	CGAGG	-GACGG	-AG	CGCG	CGAGG
Drosera_rotundifolia	CGTGT	CAACC	-AAT	GGAGG	-GATGG	-GG	CGTGT	CGTGC
Drosera_slackii	CACGTG	---	---	---	---	---	---	---
Drosophyllum_lusitanicum	???	???	???	???	???	???	???	???
Nepenthes_alata	CATGTT	---	---	---	---	---	---	---
Nepenthes_albomarginata	CATGTT	---	---	---	---	---	---	---
Nepenthes_boschiana	CATGTT	---	---	---	---	---	---	---
Nepenthes_fusca	CATGTT	---	---	---	---	---	---	---
Nepenthes_glandulifera	CATGTT	---	---	---	---	---	---	---
Nepenthes_gracillima	CATGTT	---	---	---	---	---	---	---
Nepenthes_gymnamphora	CATGTT	---	---	---	---	---	---	---
Nepenthes_hirsuta	???	???	???	???	???	???	???	???
Nepenthes_insignis	CATGTT	---	---	---	---	---	---	---
Nepenthes_macfarlanei	CATGTT	---	---	---	---	---	---	---
Nepenthes_maxima	CATGTT	---	---	---	---	---	---	---
Nepenthes_mirabilis	CATGTT	---	---	---	---	---	---	---
Nepenthes_northiana	CATGTT	---	---	---	---	---	---	---
Nepenthes_rafflesiana	CATGTT	---	---	---	---	---	---	---
Nepenthes_reinwardtiana	CATGTT	---	---	---	---	---	---	---
Nepenthes_sanguinea	CATGTT	---	---	---	---	---	---	---
Nepenthes_spectabilis	CATGTT	---	---	---	---	---	---	---
Nepenthes_singalana	???	???	???	???	???	???	???	???
Nepenthes_tentaculata	CATGTT	---	---	---	---	---	---	---
Nepenthes_tobiaca	???	???	???	???	???	???	???	???
Nepenthes_truncata	CATGTT	---	---	---	---	---	---	---
Nepenthes_ventricosa	CATGTT	---	---	---	---	---	---	---
Nepenthes_vieillardii	CATGTT	---	---	---	---	---	---	---
Triphyophyllum_peltatum	CTCGTT	---	---	---	---	---	---	---

	170	180	190	200	210	220	230	240	250
Aldrovanda_vesiculosa	?	?	?	?	?	?	?	?	?
Ancistrocladus_abbreviatus	?	?	?	?	?	?	?	?	?
Ancistrocladus_barteri	?	?	?	?	?	?	?	?	?
Ancistrocladus_benomensis	?	?	?	?	?	?	?	?	?
Ancistrocladus_cochichinensis	?	?	?	?	?	?	?	?	?
Ancistrocladus_congolensis	?	?	?	?	?	?	?	?	?
Ancistrocladus_grandiflorus	?	?	?	?	?	?	?	?	?
Ancistrocladus_guineensis	?	?	?	?	?	?	?	?	?
Ancistrocladus_hamatus	?	?	?	?	?	?	?	?	?
Ancistrocladus_heyneanus	?	?	?	?	?	?	?	?	?
Ancistrocladus_korupensis	?	?	?	?	?	?	?	?	?
Ancistrocladus_letestui	?	?	?	?	?	?	?	?	?
Dionaea_muscipula	?	?	?	?	?	?	?	?	?
Dioncophyllum_thollonii	?	?	?	?	?	?	?	?	?
Drosera_binata	?	?	?	?	?	?	?	?	?
Drosera_capensis	?	?	?	?	?	?	?	?	?
Drosera_dielisiana	?	?	?	?	?	?	?	?	?
Drosera_falconeri	?	?	?	?	?	?	?	?	?
Drosera_graminifolia	?	?	?	?	?	?	?	?	?
Drosera_hamiltonii	?	?	?	?	?	?	?	?	?
Drosera_nidiformis	?	?	?	?	?	?	?	?	?
Drosera_paleacea	?	?	?	?	?	?	?	?	?
Drosera_regia	?	?	?	?	?	?	?	?	?
Drosera_rotundifolia	?	?	?	?	?	?	?	?	?
Drosera_slackii	?	?	?	?	?	?	?	?	?
Drosophyllum_lusitanicum	?	?	?	?	?	?	?	?	?
Nepenthes_alata	?	?	?	?	?	?	?	?	?
Nepenthes_albomarginata	?	?	?	?	?	?	?	?	?
Nepenthes_boschiana	?	?	?	?	?	?	?	?	?
Nepenthes_fusca	?	?	?	?	?	?	?	?	?
Nepenthes_glandulifera	?	?	?	?	?	?	?	?	?
Nepenthes_gracillima	?	?	?	?	?	?	?	?	?
Nepenthes_gymnamphora	?	?	?	?	?	?	?	?	?
Nepenthes_hirsuta	?	?	?	?	?	?	?	?	?
Nepenthes_insignis	?	?	?	?	?	?	?	?	?
Nepenthes_macfarlanei	?	?	?	?	?	?	?	?	?
Nepenthes_maxima	?	?	?	?	?	?	?	?	?
Nepenthes_mirabilis	?	?	?	?	?	?	?	?	?
Nepenthes_northiana	?	?	?	?	?	?	?	?	?
Nepenthes_rafflesiana	?	?	?	?	?	?	?	?	?
Nepenthes_reinwardtiana	?	?	?	?	?	?	?	?	?
Nepenthes_sanguinea	?	?	?	?	?	?	?	?	?
Nepenthes_spectabilis	?	?	?	?	?	?	?	?	?
Nepenthes_tentaculata	?	?	?	?	?	?	?	?	?
Nepenthes_tobiaca	?	?	?	?	?	?	?	?	?
Nepenthes_truncata	?	?	?	?	?	?	?	?	?
Nepenthes_ventricosa	?	?	?	?	?	?	?	?	?
Nepenthes_vieillardii	?	?	?	?	?	?	?	?	?
Triphyophyllum_peltatum	?	?	?	?	?	?	?	?	?

	260	270	280	290	300	310	320	330
Aldrovanda_vesiculosa	CGACTTAAACAA-CAATC	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????
Ancistrocladus_abbreviatus	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????
Ancistrocladus_barteri	---ACTTAAATG---	CACAGGFTGCGG--	AGCCC--	CAC-CTTGGAGGCCCTC-	GTTTCTTGCTGTTTGG	-----	-----	-----
Ancistrocladus_benomensis	---ACATAAATG---	CACAGGFTGCGG--	AGCCC--	CAC-CTTGGAGGCCCTC-	GTTTCTTGCTGTTTGG	-----	-----	-----
Ancistrocladus_cochichinensis	---ACATAAATG---	CACAGGFTGCGG--	AGCCC--	CAC-CTTGGAGGCCCTC-	GTTTCTTGCTGTTTGG	-----	-----	-----
Ancistrocladus_congolensis	---ACTTAAATG---	CACAGGFTGCGG--	AGCCC--	CAC-CTTGGAGGCCCTC-	GTTTCTTGCTGTTTGG	-----	-----	-----
Ancistrocladus_grandiflorus	---ACTTAAATG---	CACAGGFTGCGG--	AGCCC--	CAC-CTTGGAGGCCCTC-	GTTTCTTGCTGTTTGG	-----	-----	-----
Ancistrocladus_guineensis	---ACTTAAATG---	CACAGGFTGCGG--	AGTCC--	CCC-CTTGGAGGCCCTC-	GCTTCTTGCTGTTTGG	-----	-----	-----
Ancistrocladus_hamatus	---ACATAAATG---	CACAGGFTGCGG--	AGCCC--	CAC-CTTGGAGGCCCTC-	GTTTCTTGCTGTTTGG	-----	-----	-----
Ancistrocladus_heyneanus	---ACTTAAATG---	CACAGGFTGCGG--	AGCCC--	CAC-CTTGGAGGCCCTC-	GTTTCTTGCTGTTTGG	-----	-----	-----
Ancistrocladus_korupensis	---ACTTAAATG---	CACAGGFTGCGG--	AGCCC--	CAC-CTTGGAGGCCCTC-	GTTTCTTGCTGTTTGG	-----	-----	-----
Ancistrocladus_letestui	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????
Ancistrocladus_robertsoniorum	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????
Dionaea_muscipula	AGCCTTAAACAA-CACCC	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????
Dionophyllum_thollonii	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????
Drosera_binata	CTAAA CAACCA -AACCC	-----	-----	-----	-----	-----	-----	-----
Drosera_capensis	CAGCA CAACCA -AACCC	-----	-----	-----	-----	-----	-----	-----
Drosera_dielisiana	CAGCA CAACCA -AACCC	-----	-----	-----	-----	-----	-----	-----
Drosera_falconeri	CAATCTTCA CAAT CT	-----	-----	-----	-----	-----	-----	-----
Drosera_graminifolia	CAACA CAACCA -AACCC	-----	-----	-----	-----	-----	-----	-----
Drosera_hamiltonii	CATCTAACCA -AACCC	-----	-----	-----	-----	-----	-----	-----
Drosera_nidiformis	CAGCA CAACCA -AACCC	-----	-----	-----	-----	-----	-----	-----
Drosera_paleacea	CAGCA CAACCA -AACCC	-----	-----	-----	-----	-----	-----	-----
Drosera_regia	CCA -CAAC -GTAACCC	-----	-----	-----	-----	-----	-----	-----
Drosera_rotundifolia	CAGCA CAACCA -AACCC	-----	-----	-----	-----	-----	-----	-----
Drosera_slackii	CAGCA CAACCA -AACCC	-----	-----	-----	-----	-----	-----	-----
Drosophyllum_lusitanicum	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????
Nepenthes_alata	---ACACAA CAAAAT -CGGG	---GAAGGA -TCT	---C-CTTATCA -AG	---GGGTCTTT	-----	-----	-----	-----
Nepenthes_albomarginata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_boschiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_fusca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_glandulifera	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gracillima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gymnamphora	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_hirsuta	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????
Nepenthes_insignis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_macfarlanei	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_maxima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_mirabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_northiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_rafflesiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_reinwardtiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_sanguinea	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_spectabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_singalana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tentaculata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tobiaca	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????	????????????????
Nepenthes_truncata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_ventricosa	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_vieillardii	-----	-----	-----	-----	-----	-----	-----	-----
Triphyophyllum_peltatum	-----	---GCA TAA CAAA -T	CGGG FTGGAG	---CCT	---C-C-CATGA	CGTAGCGT C	-----	---G

	340	350	360	370	380	390	400	410	420
Aldrovanda_vesiculosa	---	---	---	---	---	---	---	---	---
Ancistrocladus_abbreviatus	GGACGAGTCTCTCGTGATGCAA	--CGTTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	TG	AT	GGC
Ancistrocladus_barteri	GGACGAGTCTC--GTGATGCAA	--TGTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	TG	AT	GGC
Ancistrocladus_benomensis	GGACGAGTCTCTCGTGATGCAA	--CGTTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	TG	AT	GGC
Ancistrocladus_cochichinensis	GGACGAGTCTCTCGTGATGCAA	--TGTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	CG	GT	GGC
Ancistrocladus_congolensis	GGACGAGTCTC--GTGATGCAA	--CGTTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	TG	AT	GGC
Ancistrocladus_grandiflorus	GGACGAGTCTC--GTGATGCAA	--CGTTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	TG	AT	GGC
Ancistrocladus_guineensis	GGACGAGTCTC--GTGATGCAA	--CGTTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	TG	AT	GGC
Ancistrocladus_hamatus	GGACGAGTCTCTCGTGATGCAA	--CGTTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	TG	AT	GGC
Ancistrocladus_heyneanus	GGACGAGTCTCTCGTGATGCAA	--TGTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	CG	GT	GGC
Ancistrocladus_korupensis	GGACGAGTCTC--GTGATGCAA	--CGTTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	TG	AT	GGC
Ancistrocladus_letestui	????????????????????	????????????	????????	????????	????????	????????	????????	????????	????
Ancistrocladus_robertsoniorum	????????T-C--GTGATGCAA	--CGTTGTGCTA	CCTTGGGC	CAA	CGATGTGC	--A	TG	AT	GGC
Dionaea_muscipula	---	---	---	---	---	---	---	---	---
Dioncophyllum_thollonii	---	---	---	---	---	---	---	---	---
Drosera_binata	---	---	---	---	---	---	---	---	---
Drosera_capensis	---	---	---	---	---	---	---	---	---
Drosera_dielisiana	---	---	---	---	---	---	---	---	---
Drosera_falconeri	---	---	---	---	---	---	---	---	---
Drosera_graminifolia	---	---	---	---	---	---	---	---	---
Drosera_hamiltonii	---	---	---	---	---	---	---	---	---
Drosera_nidiformis	---	---	---	---	---	---	---	---	---
Drosera_paleacea	---	---	---	---	---	---	---	---	---
Drosera_regia	---	---	---	---	---	---	---	---	---
Drosera_rotundifolia	---	---	---	---	---	---	---	---	---
Drosera_slackii	---	---	---	---	---	---	---	---	---
Drosophyllum_lusitanicum	---	---	---	---	---	---	---	---	---
Nepenthes_alata	---	---	---	---	---	---	---	---	---
Nepenthes_albomarginata	---	---	---	---	---	---	---	---	---
Nepenthes_boschiana	---	---	---	---	---	---	---	---	---
Nepenthes_fusca	---	---	---	---	---	---	---	---	---
Nepenthes_glandulifera	---	---	---	---	---	---	---	---	---
Nepenthes_gracillima	---	---	---	---	---	---	---	---	---
Nepenthes_gymnamphora	---	---	---	---	---	---	---	---	---
Nepenthes_hirsuta	---	---	---	---	---	---	---	---	---
Nepenthes_insignis	---	---	---	---	---	---	---	---	---
Nepenthes_macfarlanei	---	---	---	---	---	---	---	---	---
Nepenthes_maxima	---	---	---	---	---	---	---	---	---
Nepenthes_mirabilis	---	---	---	---	---	---	---	---	---
Nepenthes_northiana	---	---	---	---	---	---	---	---	---
Nepenthes_rafflesiana	---	---	---	---	---	---	---	---	---
Nepenthes_reinwardtiana	---	---	---	---	---	---	---	---	---
Nepenthes_sanguinea	---	---	---	---	---	---	---	---	---
Nepenthes_spectabilis	---	---	---	---	---	---	---	---	---
Nepenthes_tentaculata	---	---	---	---	---	---	---	---	---
Nepenthes_tobiaca	---	---	---	---	---	---	---	---	---
Nepenthes_truncata	---	---	---	---	---	---	---	---	---
Nepenthes_ventricosa	---	---	---	---	---	---	---	---	---
Nepenthes_vieillardii	---	---	---	---	---	---	---	---	---
Triphyophyllum_peltatum	---	---	---	---	---	---	---	---	---

Aldrovanda_vesiculosa	-----TG-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_abbreviatus	T-----G-CCCTAA-CC-CAA- TAATA-AA-CAT-----	440	-----TAATA-AA-CAT-----	450	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	470
Ancistrocladus_barteri	T-----TG-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_benomensis	T-----TG-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_cochichinensis	T-----TG-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_congolensis	T-----TG-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_grandiflorus	T-----TG-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_guineensis	T-----TG-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_hamatus	T-----TG-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_heyneanus	T-----TG-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_korupensis	T-----TG-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_letestui	????????????????????????????????????????????????????????????????????????????????????	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Ancistrocladus_robertsoniorum	T-----TGC-----	430	-----TAATA-AA-CAT-----	460	-----TAATA-AA-CAT-----	490	-----	500
Dionaea_muscipula	-----	430	-----	460	-----	490	-----	500
Dioncophyllum_thollonii	-----	430	-----	460	-----	490	-----	500
Drosera_binata	-----	430	-----	460	-----	490	-----	500
Drosera_capensis	-----	430	-----	460	-----	490	-----	500
Drosera_dielisiana	-----	430	-----	460	-----	490	-----	500
Drosera_falconeri	-----	430	-----	460	-----	490	-----	500
Drosera_graminifolia	-----	430	-----	460	-----	490	-----	500
Drosera_hamiltonii	-----	430	-----	460	-----	490	-----	500
Drosera_nidiformis	-----	430	-----	460	-----	490	-----	500
Drosera_paleacea	-----	430	-----	460	-----	490	-----	500
Drosera_regia	-----	430	-----	460	-----	490	-----	500
Drosera_rotundifolia	-----	430	-----	460	-----	490	-----	500
Drosera_slackii	-----	430	-----	460	-----	490	-----	500
Drosophyllum_lusitanicum	TATCCT--TGCAACGTTAGGCATGTCCCT--GACCC--AAACAGAA-AA--CCC	430	-----	460	-----	490	-----	500
Nepenthes_alata	????????????????????????????????????????????????????????????????????????????????????	430	-----	460	-----	490	-----	500
Nepenthes_albomarginata	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_boschiana	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_fusca	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_glandulifera	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_gracillima	-----GAAACAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_gymnamphora	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_hirsuta	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_ignis	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_macfarlanei	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_maxima	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_mirabilis	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_northiana	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_rafflesiana	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_reinwardtiana	-----GAAACAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_sanguinea	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_spectabilis	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_tentaculata	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_tobiaca	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_truncata	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_ventricosa	-----GAAATAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Nepenthes_vieillardii	-----GAAACAAAATCGTCGGGGGC--ACGGCGTCTAA	430	-----	460	-----	490	-----	500
Triphyophyllum_peltatum	-----CCCCTCCGAGGT-----C-----	430	-----	460	-----	490	-----	500

Aldrovanda_vesiculosa	510	520	530	540	550	560	570	580
Ancistrocladus_abbreviatus	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_barteri	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_benomensis	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_cochichinensis	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_congolensis	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_grandiflorus	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_guineensis	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_hamatus	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_heyneanus	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_korupensis	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_letestui	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_robertsoniorum	-----	-----	-----	-----	-----	-----	-----	-----
Dionaea_muscipula	-----	-----	-----	-----	-----	-----	-----	-----
Dionocophyllum_thollonii	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_binata	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_capensis	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_dielisiana	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_falconeri	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_graminifolia	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_hamiltonii	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_nidiformis	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_paleacea	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_regia	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_rotundifolia	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_slackii	-----	-----	-----	-----	-----	-----	-----	-----
Drosophyllum_lusitanicum	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_alata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_albomarginata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_boschiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_fusca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_glandulifera	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gracillima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gymnamphora	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_hirsuta	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_insignis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_macfarlanei	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_maxima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_mirabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_northiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_rafflesiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_reinwardtiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_sanguinea	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_spectabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tentaculata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tobiaca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_truncata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_ventricosa	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_vieillardii	-----	-----	-----	-----	-----	-----	-----	-----
Triphyophyllum_peltatum	-----	-----	-----	-----	-----	-----	-----	-----

590 |-----AGCG CGGA TGCG CAAAGG ACAA TGG--A--TGCTTTGACATGC--CTTTGCCCT--T
 Aldrovanda_vesiculosa
 600 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_abbreviatus
 610 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_barteri
 620 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_benomensis
 630 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_cochichinensis
 640 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_congolensis
 650 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_grandiflorus
 660 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_guineensis
 670 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_hamatus
 680 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_heyneanus
 690 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_korupensis
 700 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_letestui
 710 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Ancistrocladus_robertsoniorum
 720 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Dionaea_muscipula
 730 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Dioncophyllum_thollonii
 740 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_binata
 750 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_capensis
 760 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_dielisiana
 770 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_falconeri
 780 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_graminifolia
 790 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_hamiltonii
 800 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_nidiformis
 810 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_paleacea
 820 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_regia
 830 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_rotundifolia
 840 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosera_slackii
 850 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Drosophyllum_lusitanicum
 860 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_alata
 870 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_albomarginata
 880 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_boschiana
 890 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_fusca
 900 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_gracillima
 910 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_gymnamphora
 920 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_hirsuta
 930 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_ignis
 940 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_macfarlanei
 950 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_maxima
 960 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_mirabilis
 970 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_northiana
 980 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_rafflesiana
 990 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_reinwardtiana
 1000 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_singalana
 1010 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_sanguinea
 1020 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_spectabilis
 1030 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_tentaculata
 1040 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_tobiaca
 1050 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_truncata
 1060 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_ventricosa
 1070 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Nepenthes_vieillardii
 1080 |-----CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--CGCG CGGAT TCGC CAAAG--
 Triphyophyllum_peltatum

	680	690	700	710	720	730	740	750
Aldrovanda_vesiculosa	CTGTCT	TGGTCT	TTGGACCA	GGGGATAGGG	GACGG	CGCGA	CGATT	GACAC
Ancistrocladus_abbreviatus	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_barteri	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_benomensis	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_cochichinensis	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_congolensis	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_grandiflorus	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_guineensis	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_hamatus	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_heyneanus	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_korupensis	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_letestui	-----	-----	-----	-----	-----	-----	-----	-----
Ancistrocladus_robertsoniorum	-----	-----	-----	-----	-----	-----	-----	-----
Dionaea_muscipula	-----	-----	-----	-----	-----	-----	-----	-----
Dioncophyllum_thollonii	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_binata	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_capensis	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_dielisiana	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_falconeri	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_graminifolia	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_hamiltonii	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_nidiformis	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_paleacea	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_regia	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_rotundifolia	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_slackii	-----	-----	-----	-----	-----	-----	-----	-----
Drosophyllum_lusitanicum	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_alata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_albomarginata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_boschiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_fusca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_glandulifera	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gracillima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gymnamphora	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_hirsuta	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_insignis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_macfarlanei	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_maxima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_mirabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_northiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_rafflesiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_reinwardtiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_sanguinea	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_spectabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_singalana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tentaculata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tobiaca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_truncata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_ventricosa	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_vieillardii	-----	-----	-----	-----	-----	-----	-----	-----
Triphyophyllum_peltatum	-----	-----	-----	-----	-----	-----	-----	-----

	760	770	780	790	800	810	820	830	840
Aldrovanda_vesiculosa	---	---	---	---	---	---	---	---	---
Ancistrocladus_abbreviatus	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAGCGTTTCA-CATTATAAACAAAC								
Ancistrocladus_barteri	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAA								
Ancistrocladus_benomensis	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAGCGTTTCA-CATTATAAACAAAC								
Ancistrocladus_cochichinensis	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAGCGTTTCA-CATTATAAACAAAC								
Ancistrocladus_congolensis	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAA								
Ancistrocladus_grandiflorus	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAA								
Ancistrocladus_guineensis	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAA								
Ancistrocladus_hamatus	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAA								
Ancistrocladus_heyneanus	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAA								
Ancistrocladus_korupensis	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAA								
Ancistrocladus_letestui	????????????????????????????????????????????????????????????								
Ancistrocladus_robertsoniorum	GCTTCCCTCGCTTCCGGTCTCGGCATGCGCGGTAGCAA								
Dionaea_muscipula	---	---	---	---	---	---	---	---	---
Diosphyllum_thollonii	---	---	---	---	---	---	---	---	---
Drosera_binata	---	---	---	---	---	---	---	---	---
Drosera_capensis	---	---	---	---	---	---	---	---	---
Drosera_dielisiana	---	---	---	---	---	---	---	---	---
Drosera_falconeri	---	---	---	---	---	---	---	---	---
Drosera_graminifolia	---	---	---	---	---	---	---	---	---
Drosera_hamiltonii	---	---	---	---	---	---	---	---	---
Drosera_nidiformis	---	---	---	---	---	---	---	---	---
Drosera_paleacea	---	---	---	---	---	---	---	---	---
Drosera_regia	---	---	---	---	---	---	---	---	---
Drosera_rotundifolia	---	---	---	---	---	---	---	---	---
Drosera_slackii	---	---	---	---	---	---	---	---	---
Drosophyllum_lusitanicum	---	---	---	---	---	---	---	---	---
Nepenthes_alata	---	---	---	---	---	---	---	---	---
Nepenthes_albomarginata	---	---	---	---	---	---	---	---	---
Nepenthes_boschiana	---	---	---	---	---	---	---	---	---
Nepenthes_fusca	---	---	---	---	---	---	---	---	---
Nepenthes_glandulifera	---	---	---	---	---	---	---	---	---
Nepenthes_gracillima	---	---	---	---	---	---	---	---	---
Nepenthes_gymnamphora	---	---	---	---	---	---	---	---	---
Nepenthes_hirsuta	---	---	---	---	---	---	---	---	---
Nepenthes_ignis	---	---	---	---	---	---	---	---	---
Nepenthes_macfarlanei	---	---	---	---	---	---	---	---	---
Nepenthes_maxima	---	---	---	---	---	---	---	---	---
Nepenthes_mirabilis	---	---	---	---	---	---	---	---	---
Nepenthes_northiana	---	---	---	---	---	---	---	---	---
Nepenthes_rafflesiana	---	---	---	---	---	---	---	---	---
Nepenthes_reinwardtiana	---	---	---	---	---	---	---	---	---
Nepenthes_sanguinea	---	---	---	---	---	---	---	---	---
Nepenthes_spectabilis	---	---	---	---	---	---	---	---	---
Nepenthes_tentaculata	---	---	---	---	---	---	---	---	---
Nepenthes_tobiaca	---	---	---	---	---	---	---	---	---
Nepenthes_truncata	---	---	---	---	---	---	---	---	---
Nepenthes_ventricosa	---	---	---	---	---	---	---	---	---
Nepenthes_vieillardii	---	---	---	---	---	---	---	---	---
Triphyophyllum_peltatum	---	---	---	---	---	---	---	---	---

	850	860	870	880	890	900	910	920
Aldrovanda_vesiculosa	---	---	---	---	---	---	GA	CT
Ancistrocladus_abbreviatus	---	---	---	---	---	---	GA	CT
Ancistrocladus_barteri	---	---	---	---	---	---	GA	CT
Ancistrocladus_benomensis	---	---	---	---	---	---	GA	CT
Ancistrocladus_cochichinensis	---	---	---	---	---	---	GA	CT
Ancistrocladus_congolensis	---	---	---	---	---	---	GA	CT
Ancistrocladus_grandiflorus	---	---	---	---	---	---	GA	CT
Ancistrocladus_guineensis	---	---	---	---	---	---	GA	CT
Ancistrocladus_hamatus	---	---	---	---	---	---	GA	CT
Ancistrocladus_heyneanus	---	---	---	---	---	---	GA	CT
Ancistrocladus_korupensis	---	---	---	---	---	---	GA	CT
Ancistrocladus_letestui	---	---	---	---	---	---	GA	CT
Ancistrocladus_robertsoniorum	---	---	---	---	---	---	GA	CT
Dionaea_muscipula	---	---	---	---	---	---	GA	CT
Dioncophyllum_thollonii	---	---	---	---	---	---	GA	CT
Drosera_binata	---	---	---	---	---	---	GA	CT
Drosera_capensis	---	---	---	---	---	---	GA	CT
Drosera_dielisiana	---	---	---	---	---	---	GA	CT
Drosera_falconeri	---	---	---	---	---	---	GA	CT
Drosera_graminifolia	---	---	---	---	---	---	GA	CT
Drosera_hamiltonii	---	---	---	---	---	---	GA	CT
Drosera_nidiformis	---	---	---	---	---	---	GA	CT
Drosera_paleacea	---	---	---	---	---	---	GA	CT
Drosera_regia	---	---	---	---	---	---	GA	CT
Drosera_rotundifolia	---	---	---	---	---	---	GA	CT
Drosera_slackii	---	---	---	---	---	---	GA	CT
Drosophyllum_lusitanicum	---	---	---	---	---	---	GA	CT
Nepenthes_alata	---	---	---	---	---	---	GA	CT
Nepenthes_albomarginata	---	---	---	---	---	---	GA	CT
Nepenthes_boschiana	---	---	---	---	---	---	GA	CT
Nepenthes_fusca	---	---	---	---	---	---	GA	CT
Nepenthes_glandulifera	---	---	---	---	---	---	GA	CT
Nepenthes_gracillima	---	---	---	---	---	---	GA	CT
Nepenthes_gymnamphora	---	---	---	---	---	---	GA	CT
Nepenthes_hirsuta	---	---	---	---	---	---	GA	CT
Nepenthes_ignis	---	---	---	---	---	---	GA	CT
Nepenthes_macfarlanei	---	---	---	---	---	---	GA	CT
Nepenthes_maxima	---	---	---	---	---	---	GA	CT
Nepenthes_mirabilis	---	---	---	---	---	---	GA	CT
Nepenthes_northiana	---	---	---	---	---	---	GA	CT
Nepenthes_affleiana	---	---	---	---	---	---	GA	CT
Nepenthes_reinwardtiana	---	---	---	---	---	---	GA	CT
Nepenthes_sanguinea	---	---	---	---	---	---	GA	CT
Nepenthes_singalana	---	---	---	---	---	---	GA	CT
Nepenthes_spectabilis	---	---	---	---	---	---	GA	CT
Nepenthes_tentaculata	---	---	---	---	---	---	GA	CT
Nepenthes_tobiaca	---	---	---	---	---	---	GA	CT
Nepenthes_truncata	---	---	---	---	---	---	GA	CT
Nepenthes_ventricosa	---	---	---	---	---	---	GA	CT
Nepenthes_vieillardii	---	---	---	---	---	---	GA	CT
Triphyophyllum_peltatum	---	---	---	---	---	---	GA	CT

	930	940	950	960	970	980	990	1,000
Aldrovanda_vesiculosa	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_abbreviatus	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_barteri	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_benomensis	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_cochichinensis	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_congolensis	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_grandiflorus	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_guineensis	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_hamatus	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_hayneanus	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_korupensis	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Ancistrocladus_letestui	????????????????????????????	????????????????????????????	????????????????????????????	????????????????????????????	????????????????????????????	????????????????????????????		
Ancistrocladus_robertsoniorum	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Dionaea_muscipula	ATATCTAGGCTCTCGCATCGATGAAGAA	CGTAGCAAAATG	CGATAAATGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCAAGT		
Dionophyllum_thollonii	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_binata	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_capensis	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_dielisiana	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_falconeri	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_graminifolia	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_hamiltonii	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_nidiformis	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_paleacea	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_regia	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_rotundifolia	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosera_slackii	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Drosophyllum_lusitanicum	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_alata	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_albomarginata	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_boschiana	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_fusca	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_glandulifera	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_gracillima	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_gymnamphora	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_hirsuta	????????????????????????????	????????????????????????????	????????????????????????????	????????????????????????????	????????????????????????????	????????????????????????????		
Nepenthes_ignis	ATATCTCGGCTATCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_macfarlanei	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_maxima	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_mirabilis	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_northiana	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_rafflesiana	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_reinwardtiana	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_sanguinea	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_spectabilis	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_tentaculata	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_tobiaca	????????????????????????????	????????????????????????????	????????????????????????????	????????????????????????????	????????????????????????????	????????????????????????????		
Nepenthes_truncata	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_ventricosa	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Nepenthes_vieillardii	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		
Triphyophyllum_peltatum	ATATCTCGGCTCTCGCATCGATGAAGAA	CGTAGCGAAATG	CGATACTTGGTGTGAATTTG	CAGAATCCC	GTGAA	CCATCGAGT		

1,010		1,020	1,030	1,040	1,050	1,060	1,070	1,080	1,090
	Aldrovanda vesiculosa								
	Ancistrocladus abbreviatus								
	Ancistrocladus_barteri								
	Ancistrocladus_benomensis								
	Ancistrocladus_cochichinensis								
	Ancistrocladus_congolensis								
	Ancistrocladus_grandiflorus								
	Ancistrocladus_guineensis								
	Ancistrocladus_hamatus								
	Ancistrocladus_heyneanus								
	Ancistrocladus_korupensis								
	Ancistrocladus_letestui								
	Ancistrocladus_robertsoniorum								
	Dionaea_muscipula								
	Dioncophyllum_thollonii								
	Drosera_binata								
	Drosera_capensis								
	Drosera_dielisiana								
	Drosera_falconeri								
	Drosera_graminifolia								
	Drosera_hamiltonii								
	Drosera_nidiformis								
	Drosera_paleacea								
	Drosera_regia								
	Drosera_rotundifolia								
	Drosera_slackii								
	Drosophyllum_lusitanicum								
	Nepenthes_alata								
	Nepenthes_albomarginata								
	Nepenthes_boschiana								
	Nepenthes_fusca								
	Nepenthes_glandulifera								
	Nepenthes_gracillima								
	Nepenthes_gymnamphora								
	Nepenthes_hirsuta								
	Nepenthes_insignis								
	Nepenthes_macfarlanei								
	Nepenthes_maxima								
	Nepenthes_mirabilis								
	Nepenthes_northiana								
	Nepenthes_rafflesiana								
	Nepenthes_reinwardtiana								
	Nepenthes_singalana								
	Nepenthes_sanguinea								
	Nepenthes_spectabilis								
	Nepenthes_tentaculata								
	Nepenthes_tobiaca								
	Nepenthes_truncata								
	Nepenthes_ventricosa								
	Nepenthes_vieillardii								
	Triphyophyllum_peltatum								

	1,100	1,110	1,120	1,130	1,140	1,150	1,160	1,170
Aldrovanda_vesiculosa	GG	TTCACTT	CAGCGGAAGT					
Ancistrocladus_abbreviatus			GCCCTACACCCTAAG	CTTTG	CATGGGGTGA			
Ancistrocladus_barteri			GCCCTACACCCTAATG	CGTAAATGGGGTGA				
Ancistrocladus_benomensis			GCCCTACACCCTAAG	CTTTG	CATGGGGTGA			
Ancistrocladus_cochichinensis			GCCCTACACCCTACA	CGTAG	CATGGGGTGA			
Ancistrocladus_congolensis			GCCCTACACCCTAAG	CGTAG	CATGGGGTGA			
Ancistrocladus_grandiflorus			GCCCTACACCCTAAG	CGTAG	CATGGGGTGA			
Ancistrocladus_guineensis			GCCCTACACCCTAAG	CGTAG	CATGGGGTGA			
Ancistrocladus_hamatus			GCCCTACACCCTAAG	CGTAG	CATGGGGTGA			
Ancistrocladus_heyneanus			GCCCTACACCCTAAG	CGTAG	CATGGGGTGA			
Ancistrocladus_korupensis			GCCCTACACCCTAAG	CGTAG	CATGGGGTGA			
Ancistrocladus_letestui			????????????????	????????	????????	????????	????????	????????
Ancistrocladus_robertsoniorum			GCCCTACACCCTAAG	CGTAG	CATGGGGTGA			
Dionaea_muscipula	AT	TCCTATCCCATCATGAGG						
Dioncophyllum_thollonii								
Drosera_binata	AA	CGGTC	CTCCTCGAGA			GCCCTCCC		ACCCCGTCGCATCGT
Drosera_capensis	TC	GCCCC	CGGTGGAGG					
Drosera_dielisiana			TGCGGGAGG					
Drosera_falconeri	AT	CGTCC	TGGTGGAGG					
Drosera_graminifolia			????????	??????	??????	??????	??????	??????
Drosera_hamiltonii	TT	CTCCC	TTGTTTAGG					
Drosera_nidiformis	TC	GCCCC	TGCGGGAGG					
Drosera_paleacea			CGGTGGAGG					
Drosera_regia	CAC	TAGTT	CTCAGTGGAGG					
Drosera_rotundifolia	TCCT	CGCCCCC	CGCGGAGG					
Drosera_slackii	TC	GCCCC	TGCGTGGAGG					
Drosophyllum_lusitanicum						ACTCC	CAACCTACATACTGCGCT	CGT
Nepenthes_alata						GCCCCG	ACCTAGCC	GCCGGCGT
Nepenthes_albomarginata						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_boschiana						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_fusca						GCCA	CCG	ACCAAGCC
Nepenthes_glandulifera						GCCCCG	ACCAAGCC	ATCGCGT
Nepenthes_gracillima						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_gymnamphora						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_hirsuta						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_ insignis						GCCCCG	ACCTAGCC	GCCC
Nepenthes_macfarlanei						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_maxima						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_mirabilis						GCCCCG	GCCGAGCC	ACCGCGT
Nepenthes_northiana						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_rafflesiana						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_reinwardtiana						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_sanguinea						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_spectabilis						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_tentaculata						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_tobiaca						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_truncata						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_ventricosa						GCCCCG	ACCAAGCC	ACCGCGT
Nepenthes_vieillardii						GCCCCG	ACCAAGCC	ACCGCGT
Triphyophyllum_peltatum						GCCCC	CC	ACCCCTTTGTCATCGT

	1,180	1,190	1,200	1,210	1,220	1,230	1,240	1,250	1,260		
Aldrovanda_vesiculosa	---	GGCGGGGGT	-GGTGGCGA	CGATGGCCCT	CCCGTGTCTC	-TCCG	---	TGGATTCCCTC	---	TG	
Ancistrocladus_abbreviatus	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_barteri	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_benomensis	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_cochichinensis	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_congolensis	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_grandiflorus	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_guineensis	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_hamatus	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_heyneanus	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_korupensis	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_letestui	???	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Ancistrocladus_robertsoniorum	---	TTGGGGCGGA	-GATTTGACTT	CCCGT	---	---	---	TGGATTCCCTC	---	AG	
Dionaea_muscipula	---	AGGGGGTGGT	-GG	---	---	---	---	TGGATTCCCTC	---	AG	
Dionocophyllum_thollonii	---	GGGGGGCGA	-A	GG	---	---	---	TGGATTCCCTC	---	AG	
Drosera_binata	---	GGCAGGTGGA	-TTATGGCCCT	CCCGT	GCC	-T	CCGTG	---	---	CG	
Drosera_capensis	---	TTTCGGTGGG	-GTATGGCCCT	CCCGT	GTCCGT	CGT	CTCGA	---	---	TG	
Drosera_dielisiana	---	TTTCGGTGGG	-GTATGGCCCT	CCCGT	GTCCGT	CGT	CTCGA	---	---	TG	
Drosera_falconeri	---	CGCAGGTGGA	-TTATGGCCCT	CCCGT	GCC	-T	CGTTT	-T	---	CG	
Drosera_graminifolia	???	TTTCGGTGGG	-GTATGGCCCT	CCCGT	GTCCGT	CGT	CTCGA	---	---	CG	
Drosera_hamiltonii	???	TTTCGGTGGG	-GTATGGCCCT	CCCGT	GTCCGT	CGT	CTCGA	---	---	CG	
Drosera_nidiformis	---	TTTCGGTGGG	-GTATGGCCCT	CCCGT	GTCCGT	CGT	CTCGA	---	---	TG	
Drosera_paleacea	---	TTTCGGTGGG	-GTATGGCCCT	CCCGT	GTCCGT	CGT	CTCGA	---	---	TG	
Drosera_regia	---	TTTCGGTGGG	-GTATGGCCCT	CCCGT	GTCCGT	CGT	CTCGA	---	---	TG	
Drosera_rotundifolia	---	TTTCGGTGGG	-GTATGGCCCT	CCCGT	GTCCGT	CGT	CTCGA	---	---	TG	
Drosera_slackii	---	TTTCGGTGGG	-GTATGGCCCT	CCCGT	GTCCGT	CGT	CTCGA	---	---	TG	
Drosophyllum_lusitanicum	---	GGAGGGAGGTG	-ATGGG	-G	CGGATG	-AGT	GGCATG	-TC	---	---	TG
Nepenthes_alata	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_albomarginata	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_boschiana	CTCTG	CATGCTT	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_fusca	CTCTG	CATGCTT	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_gracillima	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_gymnamphora	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_hirsuta	???	???	???	???	???	???	???	???	???	???	???
Nepenthes_ignis	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_macfarlanei	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_maxima	CTCTG	CATGCTT	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_mirabilis	---	CGTCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_northiana	CTCG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_rafflesiana	---	CGTCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_reinwardtiana	CTGTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_sanguinea	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_spectabilis	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_tentaculata	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_tobiaca	???	???	???	???	???	???	???	???	???	???	???
Nepenthes_truncata	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_ventricosa	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCGT	---	---	GGCGCC	---	---	TG
Nepenthes_vieillardii	CTCTG	CATGCTA	GGTTGGGCGGA	-GAGTGGCCCT	CCCTT	---	---	GGCGCC	---	---	TG
Triphyophyllum_peltatum	GCCGGG	-TG	-AAGG	-G	GGCGGAG	-ATT	GACCT	CCCGT	---	---	CGGTTGCA

	1,270	1,280	1,290	1,300	1,310	1,320	1,330	1,340
Aldrovanda_vesiculosa	CGGTTG-G-CGGAATCTGGAGC-TGGAG--CCGTTGAGCGCA-GCCGCG-TA							
Ancistrocladus_abbreviatus	TGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Ancistrocladus_barteri	TGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Ancistrocladus_benomensis	????????????????????????????????????????????????????							
Ancistrocladus_cochichinensis	TGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Ancistrocladus_congolensis	TGGTTG-GTCTAAAACTAGAGC-TCGAGG--							
Ancistrocladus_grandiflorus	TGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Ancistrocladus_guineensis	TGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Ancistrocladus_hamatus	TGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Ancistrocladus_heyneanus	TGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Ancistrocladus_korupensis	TGGTTG-GTCTAAAACTAGAGC-TCGAGG--							
Ancistrocladus_letestui	????????????????????????????????????????????????????							
Ancistrocladus_robertsoniorum	TGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Dionaea_muscipula	????????????????????????????????????????????????????							
Dioncophyllum_thollonii	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_binata	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_capensis	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_dielisiana	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_falconeri	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_graminifolia	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_hamiltonii	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_nidiformis	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_paleacea	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_regia	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_rotundifolia	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosera_slackii	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Drosophyllum_lusitanicum	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_alata	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_albomarginata	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_boschiana	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_fusca	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_glandulifera	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_gracillima	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_gymnamphora	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_hirsuta	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_insignis	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_macfarlanei	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_maxima	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_mirabilis	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_northiana	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_rafflesiana	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_reinwardtiana	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_sanguinea	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_spectabilis	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_tentaculata	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_tobiaca	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_truncata	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_ventricosa	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Nepenthes_vieillardii	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							
Triphophyllum_peltatum	CGGTTG-GTCTAAAACTAGAGC-TCGGGG--							

	1,350	1,360	1,370	1,380	1,390	1,400	1,410	1,420																			
<i>Aldrovanda_vesiculosa</i>	---	TGG	TG	G	TTGGG	--C-	GAGG	-GAA	-A	---	CT	---	CC	---	CGA	-T	CGT	?	?	?	?	?	?	?	?	?	?
<i>Ancistrocladus_abbreviatus</i>	---	GTTGG	AC	G	ACGAG	-GCC																					
<i>Ancistrocladus_barteri</i>	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???
<i>Ancistrocladus_benomensis</i>	---	GTTGG	AC	G	ACGAG	-GCC																					
<i>Ancistrocladus_cochichinensis</i>	---	GTTGG	AC	G	ACGAG	-GCC																					
<i>Ancistrocladus_congolensis</i>	---	GTTGG	AC	G	ACGAG	-GCC																					
<i>Ancistrocladus_grandiflorus</i>	---	GTTGG	AC	G	ACGAG	-GCC																					
<i>Ancistrocladus_guineensis</i>	---	GTTGG	AC	G	ACGAG	-GCC																					
<i>Ancistrocladus_hamatus</i>	---	GTTGG	AC	G	ATGAG	-GCG																					
<i>Ancistrocladus_hayneanus</i>	---	GTTGG	AC	G	ACGAG	-GCC																					
<i>Ancistrocladus_korupensis</i>	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????
<i>Ancistrocladus_letestui</i>	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????
<i>Dionaea_muscipula</i>	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????
<i>Dionocophyllum_thollonii</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Drosera_binata</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Drosera_capensis</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Drosera_dielisiana</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Drosera_falconeri</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Drosera_graminifolia</i>	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????
<i>Drosera_hamiltonii</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Drosera_nidiformis</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Drosera_palaacea</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Drosera_rotundifolia</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Drosera_slackii</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Drosophyllum_lusitanicum</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_alata</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_albomarginata</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_boschiana</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_fusca</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_glandulifera</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_gracillima</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_gymnamphora</i>	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???
<i>Nepenthes_hirsuta</i>	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???
<i>Nepenthes_insignis</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_macfarlanei</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_maxima</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_mirabilis</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_northiana</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_afflelsiana</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_reinwardtiana</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_sanguinea</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_spectabilis</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_tentaculata</i>	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????
<i>Nepenthes_tobiaca</i>	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????	?????
<i>Nepenthes_truncata</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_ventricosa</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Nepenthes_vieillardii</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG
<i>Triphophyllum_peltatum</i>	---	GTTGG	TT	A	GCGAG	--TC	CTGAA	CGG	CA	-AG	CGT	TT	A	CGAC	CT	TGT	CA	CG	T	CC	---	GT	---	TTG	CCAA	C	TTG

	1,430	1,440	1,450	1,460	1,470	1,480	1,490	1,500	1,510
Aldrovanda_vesiculosa	???	???	???	???	???	???	???	???	???
Ancistrocladus_abbreviatus	---	---	---	---	---	---	---	---	---
Ancistrocladus_barteri	---	---	---	---	---	---	---	---	---
Ancistrocladus_benomensis	---	---	---	---	---	---	---	---	---
Ancistrocladus_cochichinensis	---	---	---	---	---	---	---	---	---
Ancistrocladus_congolensis	---	---	---	---	---	---	---	---	---
Ancistrocladus_grandiflorus	---	---	---	---	---	---	---	---	---
Ancistrocladus_guineensis	---	---	---	---	---	---	---	---	---
Ancistrocladus_hamatus	---	---	---	---	---	---	---	---	---
Ancistrocladus_heyneanus	---	---	---	---	---	---	---	---	---
Ancistrocladus_korupensis	---	---	---	---	---	---	---	---	---
Ancistrocladus_letestui	---	---	---	---	---	---	---	---	---
Ancistrocladus_robertsoniorum	---	---	---	---	---	---	---	---	---
Dionaea_muscipula	---	---	---	---	---	---	---	---	---
Dioncophyllum_thollonii	---	---	---	---	---	---	---	---	---
Drosera_binata	---	---	---	---	---	---	---	---	---
Drosera_capensis	---	---	---	---	---	---	---	---	---
Drosera_dielisiana	---	---	---	---	---	---	---	---	---
Drosera_falconeri	---	---	---	---	---	---	---	---	---
Drosera_graminifolia	---	---	---	---	---	---	---	---	---
Drosera_hamiltonii	---	---	---	---	---	---	---	---	---
Drosera_nidiformis	---	---	---	---	---	---	---	---	---
Drosera_paleacea	---	---	---	---	---	---	---	---	---
Drosera_regia	---	---	---	---	---	---	---	---	---
Drosera_rotundifolia	---	---	---	---	---	---	---	---	---
Drosera_slackii	---	---	---	---	---	---	---	---	---
Drosophyllum_lusitanicum	---	---	---	---	---	---	---	---	---
Nepenthes_alata	---	---	---	---	---	---	---	---	---
Nepenthes_albomarginata	---	---	---	---	---	---	---	---	---
Nepenthes_boschiana	---	---	---	---	---	---	---	---	---
Nepenthes_fusca	---	---	---	---	---	---	---	---	---
Nepenthes_glandulifera	---	---	---	---	---	---	---	---	---
Nepenthes_gracillima	---	---	---	---	---	---	---	---	---
Nepenthes_gymnamphora	---	---	---	---	---	---	---	---	---
Nepenthes_hirsuta	---	---	---	---	---	---	---	---	---
Nepenthes_insignis	---	---	---	---	---	---	---	---	---
Nepenthes_macfarlanei	---	---	---	---	---	---	---	---	---
Nepenthes_maxima	---	---	---	---	---	---	---	---	---
Nepenthes_mirabilis	---	---	---	---	---	---	---	---	---
Nepenthes_northiana	---	---	---	---	---	---	---	---	---
Nepenthes_rafflesiana	---	---	---	---	---	---	---	---	---
Nepenthes_reinwardtiana	---	---	---	---	---	---	---	---	---
Nepenthes_sanguinea	---	---	---	---	---	---	---	---	---
Nepenthes_spectabilis	---	---	---	---	---	---	---	---	---
Nepenthes_singalana	---	---	---	---	---	---	---	---	---
Nepenthes_tentaculata	---	---	---	---	---	---	---	---	---
Nepenthes_tobiaca	---	---	---	---	---	---	---	---	---
Nepenthes_truncata	---	---	---	---	---	---	---	---	---
Nepenthes_ventricosa	---	---	---	---	---	---	---	---	---
Nepenthes_vieillardii	---	---	---	---	---	---	---	---	---
Triphyophyllum_peltatum	---	---	---	---	---	---	---	---	---

	1,520	1,530	1,540	1,550	1,560	1,570	1,580	1,590
Aldrovanda_vesiculosa	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_abbreviatus	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_barteri	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_benomensis	CATATGACCC	TATTGAGCT	CATAAAG?	????????	????????	????????	????????	????????
Ancistrocladus_cochichinensis	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_congolensis	CATACGACCC	TATTGAGCT	CATAAAGCA	AAGGCT	CCAA	CTCT	-----	-----
Ancistrocladus_grandiflorus	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_guineensis	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_hamatus	CATACGACCC	TATTGAGCG	CATAAAGCA	AAGGCT	CCAA	CTCT	-----	-----
Ancistrocladus_heyneanus	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_korupensis	CATATGACCC	TATTGAGCG	CATAAAGCA	AAGGCT	CCAA	CTCT	-----	-----
Ancistrocladus_letestui	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_robertsoniorum	CATACGACCC	TATTGAGCG	CATAAAGCA	AAGGCT	CCAA	CTCT	-----	-----
Dionaea_muscipula	????????	????????	????????	????????	????????	????????	????????	????????
Dioncophyllum_thollonii	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_binata	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_capensis	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_dielisiana	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_falconeri	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_graminifolia	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_hamiltonii	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_nidiformis	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_paleacea	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_regia	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_rotundifolia	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_slackii	????????	????????	????????	????????	????????	????????	????????	????????
Drosophyllum_lusitanicum	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_alata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_albomarginata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_boschiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_fusca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_glandulifera	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gracillima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gymnamphora	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_hirsuta	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_ignis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_macfarlanei	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_maxima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_mirabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_northiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_rafflesiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_reinwardtiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_singalana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_spectabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tentaculata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tobiaca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_truncata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_ventricosa	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_vieillardii	-----	-----	-----	-----	-----	-----	-----	-----
Triphyophyllum_peltatum	-----	-----	-----	-----	-----	-----	-----	-----

	1,600	1,610	1,620	1,630	1,640	1,650	1,660	1,670	1,680
Aldrovanda_vesiculosa	???	???	???	???	???	???	???	???	???
Ancistrocladus_abbreviatus	???	???	???	???	???	???	???	???	???
Ancistrocladus_barteri	???	???	???	???	???	???	???	???	???
Ancistrocladus_benomensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_cochichinensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_congolensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_grandiflorus	???	???	???	???	???	???	???	???	???
Ancistrocladus_guineensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_hamatus	???	???	???	???	???	???	???	???	???
Ancistrocladus_heyneanus	???	???	???	???	???	???	???	???	???
Ancistrocladus_korupensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_letestui	???	???	???	???	???	???	???	???	???
Ancistrocladus_robertsoniorum	???	???	???	???	???	???	???	???	???
Dionaea_muscipula	???	???	???	???	???	???	???	???	???
Dioncophyllum_thollonii	???	???	???	???	???	???	???	???	???
Drosera_binata	???	???	???	???	???	???	???	???	???
Drosera_capensis	???	???	???	???	???	???	???	???	???
Drosera_dielisiana	???	???	???	???	???	???	???	???	???
Drosera_falconeri	???	???	???	???	???	???	???	???	???
Drosera_graminifolia	???	???	???	???	???	???	???	???	???
Drosera_hamiltonii	???	???	???	???	???	???	???	???	???
Drosera_nidiformis	???	???	???	???	???	???	???	???	???
Drosera_paleacea	???	???	???	???	???	???	???	???	???
Drosera_regia	???	???	???	???	???	???	???	???	???
Drosera_rotundifolia	???	???	???	???	???	???	???	???	???
Drosera_slackii	???	???	???	???	???	???	???	???	???
Drosophyllum_lusitanicum	???	???	???	???	???	???	???	???	???
Nepenthes_alata	???	???	???	???	???	???	???	???	???
Nepenthes_albomarginata	???	???	???	???	???	???	???	???	???
Nepenthes_boschiana	???	???	???	???	???	???	???	???	???
Nepenthes_fusca	???	???	???	???	???	???	???	???	???
Nepenthes_glandulifera	???	???	???	???	???	???	???	???	???
Nepenthes_gracillima	???	???	???	???	???	???	???	???	???
Nepenthes_gymnamphora	???	???	???	???	???	???	???	???	???
Nepenthes_hirsuta	???	???	???	???	???	???	???	???	???
Nepenthes_ignis	???	???	???	???	???	???	???	???	???
Nepenthes_macfarlanei	???	???	???	???	???	???	???	???	???
Nepenthes_maxima	???	???	???	???	???	???	???	???	???
Nepenthes_northiana	???	???	???	???	???	???	???	???	???
Nepenthes_rafflesiana	???	???	???	???	???	???	???	???	???
Nepenthes_reinwardtiana	???	???	???	???	???	???	???	???	???
Nepenthes_sanguinea	???	???	???	???	???	???	???	???	???
Nepenthes_singalana	???	???	???	???	???	???	???	???	???
Nepenthes_spectabilis	???	???	???	???	???	???	???	???	???
Nepenthes_tentaculata	???	???	???	???	???	???	???	???	???
Nepenthes_tobiaca	???	???	???	???	???	???	???	???	???
Nepenthes_truncata	???	???	???	???	???	???	???	???	???
Nepenthes_ventricosa	???	???	???	???	???	???	???	???	???
Nepenthes_vieillardii	???	???	???	???	???	???	???	???	???
Triphyophyllum_peltatum	???	???	???	???	???	???	???	???	???

	1,690	1,700	1,710	1,720	1,730	1,740	1,750	1,760
Aldrovanda_vesiculosa	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_abbreviatus	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_barteri	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_benomensis	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_cochichinensis	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_congolensis	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_grandiflorus	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_guineensis	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_hamatus	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_heyneanus	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_korupensis	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_letestui	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_robertsoniorum	????????	????????	????????	????????	????????	????????	????????	????????
Dionaea_muscipula	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_binata	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_capensis	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_dielisiana	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_falconeri	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_graminifolia	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_nidiformis	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_paleacea	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_regia	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_rotundifolia	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_slackii	????????	????????	????????	????????	????????	????????	????????	????????
Drosophyllum_lusitanicum	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_alata	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_albomarginata	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_boschiana	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_fusca	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_glandulifera	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_gracillima	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_gymnamphora	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_hirsuta	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_ignis	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_macfarlanei	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_maxima	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_mirabilis	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_northiana	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_rafflesiana	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_reinwardtiana	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_sanguinea	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_singalana	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_spectabilis	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_tentaculata	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_tobiaca	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_truncata	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_ventricosa	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_vieillardii	????????	????????	????????	????????	????????	????????	????????	????????
Triphyophyllum_peltatum	????????	????????	????????	????????	????????	????????	????????	????????

	1,850	1,860	1,870	1,880	1,890	1,900	1,910	1,920	1,930
Aldrovanda_vesiculosa	ATTATGATTTGGAAGTTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Ancistrocladus_abbreviatus	????????????????????????????????????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_barteri	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Ancistrocladus_benomensis	????????AGTCCATCTAGGGCAA	-TGTTC	CGGATCTA	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	-TT
Ancistrocladus_cochichinensis	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Ancistrocladus_congolensis	????????????????????????????????????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_grandiflorus	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Ancistrocladus_guineensis	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Ancistrocladus_hamatus	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Ancistrocladus_heyneanus	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Ancistrocladus_korupensis	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Ancistrocladus_letestui	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Ancistrocladus_robertsoniorum	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Dionaea_muscipula	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Dionophyllum_thollonii	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Drosera_binata	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Drosera_capensis	????????????????????????????????????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_dielisiana	????????????????????????????????????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_falconeri	????????????????????????????????????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_graminifolia	????????????????????????????????????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_nidiformis	????????????????????????????????????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_paleacea	????????????????????????????????????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_regia	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Drosera_rotundifolia	????????????????????????????????????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_slackii	????????????????????????????????????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosophyllum_lusitanicum	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_alata	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_albomarginata	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_boschiana	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_fusca	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_glandulifera	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_gracillima	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_gymnamphora	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_hirsuta	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_ignis	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_macfarlanei	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_maxima	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_mirabilis	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_northiana	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_rafflesiana	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_reinwardtiana	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_singalana	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_sanguinea	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_spectabilis	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_tentaculata	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_tobiaca	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_truncata	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_ventricosa	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Nepenthes_vieillardii	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	
Triphyophyllum_peltatum	ATTATGATCTGGAATTCGATCCAGGGCAAAGTGTTCGAAATCTAT	-FATGACAT	-AGCTGT	CAGGCG	CGCC	AGGAC	CGCAT	-TGT	

	1,940	1,950	1,960	1,970	1,980	1,990	2,000	2,010	
Aldrovanda_vesiculosa	TTT-ATTAT-	-----AA	TAAAG	CCTTTT	-----GAT	GGAAAAG	CCTT	CTTTGTGGAA	CTTTAG
Ancistrocladus_abbreviatus	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_barteri	-AT-AGT	ATAAT	AAAT	-----AA	GAAA	CCTTTT	CTGG	CTTTGGAT	TGA
Ancistrocladus_benomensis	-AT-AGT	ATAAT	AA-T	-----AA	GAAA	CCTTTT	CTGG	CTTTGGAT	TGA
Ancistrocladus_cochichinensis	-AT-AGT	ATAAT	AAAT	-----AA	AAAA	CCTTTT	CTGG	CTTTGGAT	TGA
Ancistrocladus_congolensis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_grandiflorus	-AT-AGT	ATAAT	AAAA	-----AA	AAAA	CCT	-----CT	GG	-T
Ancistrocladus_guineensis	-AT-AGT	ATAAT	AAAT	-----AA	GAAA	CCTTTT	CTGG	CTTTGGAT	TGA
Ancistrocladus_hamatus	-AT-AGT	ATAAT	AAAT	-----AA	AAAA	CCTTTT	CTGG	CTTTGGAT	TGA
Ancistrocladus_heyneanus	-AT-AGT	ATAAT	AAAT	-----AA	GAAA	CCTTTT	CTGG	CTTTGGAT	TGA
Ancistrocladus_korupensis	-AT-AGT	ATAAT	AAAT	-----AA	GAAA	CCTTTT	CTGG	CTTTGGAT	TGA
Ancistrocladus_letestui	-AT-AGT	ATAAT	AAAT	-----AA	GAAA	CCTTTT	CTGG	CTTTGGAT	TGA
Ancistrocladus_robertsoniorum	-AT-AGT	ATAAT	AAAT	-----AA	GAAA	CCTTTT	CTGG	CTTTGGAT	TGA
Dionaea_muscipula	TC-	-T-AT	-----AA	AAAA	CTTT	G	-----GAT	GT	AAA
Dionophyllum_thollonii	TT-AA	TAAAT	ATAAT	AAAT	-----AA	GAAA	CCTTTT	CTGG	CTTTGGAT
Drosera_binata	-A	TTAG	-----AA	TAAA	CTTT	T	-----GAT	GT	AAA
Drosera_capensis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_dielisiana	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_falconeri	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_graminifolia	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_hamiltonii	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_nidiformis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_paleacea	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_regia	TTG-ATT	CT	-----AA	TAAA	CTTT	T	-----GAT	GT	AAA
Drosera_rotundifolia	TC-	-ATT	CT	-----AA	TAAA	CTTT	T	-----GAT	GT
Drosera_slackii	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosophyllum_lusitanicum	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_alata	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_albomarginata	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_boschiana	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_fusca	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_glandulifera	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_gracillima	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_gymnamphora	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_hirsuta	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_ignis	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_macfarlanei	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_maxima	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_northiana	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_affleiana	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_reinwardtiana	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_singalana	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_sanguinea	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_spectabilis	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_tentaculata	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_tobiaca	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_truncata	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_ventricosa	TT-	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Nepenthes_vieillardii	TTT-A	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT
Triphyophyllum_peltatum	TTT-A	-----A	CTAT	-----AA	AAAA	TA	TTT	CTGG	CTT

	2,020	2,030	2,040	2,050	2,060	2,070	2,080	2,090	2,100
Aldrovanda_vesiculosa	---TTCCCT	---ACTAT	CCTAA	CCTAA	TTA	---CCTAA	TTGAA	TTT	---CATTTT
Ancistrocladus_abbreviatus	????????	??????	??????	??????	??????	??????	??????	??????	??????
Ancistrocladus_barteri	CCTATT	CCTATT	CCTATT	CCTATT	CCTATT	CCTATT	CCTATT	CCTATT	CCTATT
Ancistrocladus_benomensis	CCCAT	TTT	TTA	T	CT	CAATT	---	---	---
Ancistrocladus_cochichinensis	CCTAT	TCCTA	T	CT	CAATT	---	---	---	---
Ancistrocladus_congolensis	????????	??????	??????	??????	??????	??????	??????	??????	??????
Ancistrocladus_grandiflorus	---CTAT	TT	CTA	T	CT	CA	---	---	---
Ancistrocladus_guineensis	CCTATT	CTAT	CT	CAATT	---	---	---	---	---
Ancistrocladus_hamatus	CCTAT	TCCTA	T	CT	CAATT	---	---	---	---
Ancistrocladus_heyneanus	CCTAT	TT	CTA	T	CT	CAATT	---	---	---
Ancistrocladus_korupensis	CCTAT	TT	CTA	T	CT	CAATT	---	---	---
Ancistrocladus_letestui	CCTAT	TT	CTA	T	CT	CAATT	---	---	---
Ancistrocladus_robertsoniorum	CCTAT	TCCTA	T	CT	CAATT	---	---	---	---
Dionaea_muscipula	---TTCCCT	---	---AAT	TAT	CGAA	CAAAA	GAAT	GAAT	---AAG
Dionophyllum_thollonii	CCTAT	TCCTA	T	CT	CAATT	---	---	---	---
Drosera_binata	---TTT	CT	---	---AATA	---	---AAAT	GAAT	GAAT	TTA
Drosera_capensis	????????	??????	??????	??????	??????	??????	??????	??????	??????
Drosera_dielisiana	????????	??????	??????	??????	??????	??????	??????	??????	??????
Drosera_falconeri	????????	??????	??????	??????	??????	??????	??????	??????	??????
Drosera_graminifolia	????????	??????	??????	??????	??????	??????	??????	??????	??????
Drosera_nidiformis	????????	??????	??????	??????	??????	??????	??????	??????	??????
Drosera_paleacea	????????	??????	??????	??????	??????	??????	??????	??????	??????
Drosera_regia	---TTCCCT	---	---AAT	---	---AA	---CTAAT	CAAT	TAT	CTA
Drosera_rotundifolia	---TTCCCT	---	---AAT	---	---AA	---CTAAT	CAAT	TAT	CTA
Drosera_slackii	????????	??????	??????	??????	??????	??????	??????	??????	??????
Drosophyllum_lusitanicum	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_alata	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_albomarginata	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_boschiana	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_fusca	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_glandulifera	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_gracillima	TGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_gymnamphora	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_hirsuta	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_ignis	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_macfarlanei	TGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_maxima	TGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_mirabilis	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_northiana	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_rafflesiana	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_reinwardtiana	TGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_sanguinea	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_singalana	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_spectabilis	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_tentaculata	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_tobiaca	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_truncata	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_ventricosa	CGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Nepenthes_vieillardii	TGTAT	TCCTA	T	CT	CAATT	---	---	---	---
Triphyophyllum_peltatum	CCTAT	TCCTA	T	CT	CAATT	---	---	---	---

	2,110	2,120	2,130	2,140	2,150	2,160	2,170	2,180
Aldrovanda_vesiculosa	???	???	???	???	???	???	???	???
Ancistrocladus_abbreviatus	???	???	???	???	???	???	???	???
Ancistrocladus_barteri	???	???	???	???	???	???	???	???
Ancistrocladus_benomensis	???	???	???	???	???	???	???	???
Ancistrocladus_cochichinensis	???	???	???	???	???	???	???	???
Ancistrocladus_congolensis	???	???	???	???	???	???	???	???
Ancistrocladus_grandiflorus	???	???	???	???	???	???	???	???
Ancistrocladus_guineensis	???	???	???	???	???	???	???	???
Ancistrocladus_hamatus	???	???	???	???	???	???	???	???
Ancistrocladus_heyneanus	???	???	???	???	???	???	???	???
Ancistrocladus_korupensis	???	???	???	???	???	???	???	???
Ancistrocladus_letestui	???	???	???	???	???	???	???	???
Ancistrocladus_robertsonianorum	???	???	???	???	???	???	???	???
Dionaea_muscipula	GT--ATTCT	-----	-----	-----	AAAAG	-----	AAATGAAATAATCGAT	-----
Dioncophyllum_thollonii	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_binata	GTAATTT	-----	-----	-----	-----	-----	-----	-----
Drosera_capensis	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_dielisiana	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_falconeri	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_graminifolia	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_hamiltonii	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_nidiformis	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_paleacea	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_regia	CTATACTTCTAATTAATAATAATAATAATTC	-----	-----	-----	-----	-----	-----	-----
Drosera_rotundifolia	-----	-----	-----	-----	-----	-----	-----	-----
Drosera_slackii	-----	-----	-----	-----	-----	-----	-----	-----
Drosophyllum_lusitanicum	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_alata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_albomarginata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_boschiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_fusca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_glandulifera	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gracillima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gymnamphora	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_hirsuta	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_insignis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_macfarlanei	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_maxima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_mirabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_northiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_rafflesiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_reinwardtiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_singalana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_sanguinea	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_spectabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tentaculata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tobiaca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_truncata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_ventricosa	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_vieillardii	-----	-----	-----	-----	-----	-----	-----	-----
Triphyophyllum_peltatum	-----	-----	-----	-----	-----	-----	-----	-----

	2,190	2,200	2,210	2,220	2,230	2,240	2,250	2,260
Aldrovanda_vesiculosa	???	???	???	???	???	???	???	???
Ancistrocladus_abbreviatus	???	???	???	???	???	???	???	???
Ancistrocladus_barteri	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Ancistrocladus_benomensis	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Ancistrocladus_cochichinensis	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Ancistrocladus_congolensis	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Ancistrocladus_grandiflorus	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Ancistrocladus_guineensis	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Ancistrocladus_hamatus	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Ancistrocladus_heyneanus	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Ancistrocladus_korupensis	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Ancistrocladus_letestui	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Ancistrocladus_robertsoniorum	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Dionaea_muscipula	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Dionophyllum_thollonii	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_binata	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_capensis	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_dielisiana	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_falconeri	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_graminifolia	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_hamiltonii	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_nidiformis	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_paleacea	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_regia	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_rotundifolia	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosera_slackii	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Drosophyllum_lusitanicum	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_alata	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_albomarginata	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_boschiana	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_fusca	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_glandulifera	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_gracillima	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_gymnamphora	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_hirsuta	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_ignis	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_macfarlanei	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_maxima	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_mirabilis	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_northiana	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_rafflesiana	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_reinwardtiana	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_sanguinea	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_singalana	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_spectabilis	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_tentaculata	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_tobiaca	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_truncata	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_ventricosa	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Nepenthes_vieillardii	---	AGAA	---	AGAA	---	AGAA	---	AGAA
Triphophyllum_peltatum	---	AGAA	---	AGAA	---	AGAA	---	AGAA

Aldrovanda_vesiculosa	2,270	2,280	2,290	2,300	2,310	2,320	2,330	2,340	2,350
Ancistrocladus_abbreviatus	???	???	???	???	???	???	???	???	???
Ancistrocladus_barteri	???	???	???	???	???	???	???	???	???
Ancistrocladus_benomensis	TTT	-----	CCAAATCGCTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CCAT	-----
Ancistrocladus_cochichinensis	TTT	-----	CCAAATCGCTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CCAT	-----
Ancistrocladus_congolensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_grandiflorus	TTT	-----	CCAAATCGCTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CCAT	-----
Ancistrocladus_guineensis	TTT	-----	CCAAATCGCTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CCAT	-----
Ancistrocladus_hamatus	TTT	-----	CCAAATCGCTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CCAT	-----
Ancistrocladus_heyneanus	TTT	-----	CCAAATCGCTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CCAT	-----
Ancistrocladus_korupensis	TTT	-----	CCAAATCGCTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CCAT	-----
Ancistrocladus_letestui	TTT	-----	CCAAATCGCTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CCAT	-----
Ancistrocladus_robertsoniorum	TTT	-----	CCAAATCGCTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CCAT	-----
Dionaea_muscipula	CTCGA	-----	TTGAAATCGCGTAAAGCAGCA	TTA	-----	CTAAGAAAGATCTCGGTAT	CTCTTTTCTA	TTTTCA	TTTTCGAAAT
Dionophyllum_thollonii	TTT	-----	CCAAATCGCTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CTAT	-----
Drosera_binata	-----	-----	CTAAATCGCGTGAGCAGCTA	TTA	-----	CTAAGAAAGATCTCGGTAT	CTCTTTTCTA	TTTTCA	TTTTCGAAAT
Drosera_capensis	???	???	???	???	???	???	???	???	???
Drosera_dielisiana	???	???	???	???	???	???	???	???	???
Drosera_falconeri	???	???	???	???	???	???	???	???	???
Drosera_graminifolia	???	???	???	???	???	???	???	???	???
Drosera_hamiltonii	???	???	???	???	???	???	???	???	???
Drosera_nidiformis	???	???	???	???	???	???	???	???	???
Drosera_paleacea	???	???	???	???	???	???	???	???	???
Drosera_regia	-----	-----	ATAATCGCGTGAGCAGCTA	TTA	-----	CTAAGAAAGATCTCGGTAT	CTTTTTTCTA	TTTTCA	TTTTCGAAAT
Drosera_rotundifolia	-----	-----	CTAAATCTCAATCGTA	CGAGCAGCA	TTA	-----	ATAAGCAAGATCTCGGTAT	CTTTTCTA	TTTTCGAAAT
Drosera_slackii	???	???	???	???	???	???	???	???	???
Drosophyllum_lusitanicum	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_alata	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_albomarginata	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_boschiana	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_fusca	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_glandulifera	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_gracillima	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_gymnamphora	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_hirsuta	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_ignis	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_macfarlanei	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_maxima	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_mirabilis	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_northiana	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_rafflesiana	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_reinwardtiana	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_sanguinea	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_singalana	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_spectabilis	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_tentaculata	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_tobiaca	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_truncata	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_ventricosa	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Nepenthes_vieillardii	-----	-----	CCAAATCCAAATCCGAG	CGAGCAGTCA	TTAGTCA	TTA	CTAAGAAAGATCTCGGTAT	-----	CTAT
Triphyophyllum_peltatum	TTT	-----	CCAAATCCGCGTGAGCAGTCA	TTA	-----	CTAAGAAAGATCTCGGTAT	-----	CTAT	-----

	2,360	2,370	2,380	2,390	2,400	2,410	2,420	2,430
Aldrovanda_vesiculosa	???	???	???	???	???	???	???	???
Ancistrocladus_abbreviatus	???	???	???	???	???	???	???	???
Ancistrocladus_barteri	TC	TC	TC	TC	TC	TC	TC	TC
Ancistrocladus_benomensis	TC	TC	TC	TC	TC	TC	TC	TC
Ancistrocladus_cochichinensis	TC	TC	TC	TC	TC	TC	TC	TC
Ancistrocladus_congolensis	TC	TC	TC	TC	TC	TC	TC	TC
Ancistrocladus_grandiflorus	TC	TC	TC	TC	TC	TC	TC	TC
Ancistrocladus_guineensis	TC	TC	TC	TC	TC	TC	TC	TC
Ancistrocladus_hamatus	TC	TC	TC	TC	TC	TC	TC	TC
Ancistrocladus_heyneanus	TC	TC	TC	TC	TC	TC	TC	TC
Ancistrocladus_korupensis	TC	TC	TC	TC	TC	TC	TC	TC
Ancistrocladus_letestui	TC	TC	TC	TC	TC	TC	TC	TC
Ancistrocladus_robertsonianorum	TC	TC	TC	TC	TC	TC	TC	TC
Dionaea_muscipula	TCGAAATGAAAT	TC	TC	TC	TC	TC	TC	TC
Dioncophyllum_thollonii	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_binata	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_capensis	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_dielisiana	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_falconeri	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_graminifolia	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_hamiltonii	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_nidiformis	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_paleacea	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_regia	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_rotundifolia	TC	TC	TC	TC	TC	TC	TC	TC
Drosera_slackii	TC	TC	TC	TC	TC	TC	TC	TC
Drosophyllum_lusitanicum	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_alata	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_albomarginata	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_boschiana	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_fusca	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_glandulifera	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_gracillima	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_gymnamphora	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_hirsuta	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_insignis	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_macfarlanei	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_maxima	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_mirabilis	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_northiana	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_rafflesiana	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_reinwardtiana	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_sanguinea	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_spectabilis	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_tentaculata	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_tobiaca	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_truncata	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_ventricosa	TC	TC	TC	TC	TC	TC	TC	TC
Nepenthes_vieillardii	TC	TC	TC	TC	TC	TC	TC	TC
Triphyophyllum_peltatum	TC	TC	TC	TC	TC	TC	TC	TC

	2,440	2,450	2,460	2,470	2,480	2,490	2,500	2,510	2,520
Aldrovanda_vesiculosa	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_abbreviatus	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_barteri	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_benomensis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_cochichinensis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_congolensis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_grandiflorus	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_guineensis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_hamatus	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_heyneanus	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_korupensis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_letestui	????????	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_robertsoniorum	????????	????????	????????	????????	????????	????????	????????	????????	????????
Dionaea_muscipula	????????	????????	????????	????????	????????	????????	????????	????????	????????
Dionophyllum_thollonii	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_binata	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_capensis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_dielisiana	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_falconeri	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_graminifolia	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_hamiltonii	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_nidiformis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_paleacea	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_regia	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_rotundifolia	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_slackii	????????	????????	????????	????????	????????	????????	????????	????????	????????
Drosophyllum_lusitanicum	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_alata	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_albomarginata	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_boschiana	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_fusca	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_glandulifera	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_gracillima	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_gymnamphora	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_hirsuta	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_insignis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_macfarlanei	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_maxima	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_mirabilis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_northiana	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_rafflesiana	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_reinwardtiana	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_sanguinea	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_spectabilis	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_tentaculata	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_tobiaca	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_truncata	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_ventricosa	????????	????????	????????	????????	????????	????????	????????	????????	????????
Nepenthes_vieillardii	????????	????????	????????	????????	????????	????????	????????	????????	????????
Triphyophyllum_peltatum	????????	????????	????????	????????	????????	????????	????????	????????	????????

	2,610	2,620	2,630	2,640	2,650	2,660	2,670	2,680
Aldrovanda_vesiculosa	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_abbreviatus	????????	????????	????????	????????	????????	????????	????????	????????
Ancistrocladus_barteri	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Ancistrocladus_benomensis	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Ancistrocladus_cochichinensis	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Ancistrocladus_congolensis	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Ancistrocladus_grandiflorus	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Ancistrocladus_guineensis	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Ancistrocladus_hamatus	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Ancistrocladus_heyneanus	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Ancistrocladus_korupensis	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Ancistrocladus_letestui	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Ancistrocladus_robertsoniorum	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--	AAATTC--
Dionaea_muscipula	GATTCCT--	GATTCCT--	GATTCCT--	GATTCCT--	GATTCCT--	GATTCCT--	GATTCCT--	GATTCCT--
Dionophyllum_thollonii	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_binata	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_capensis	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_dielisiana	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_falconeri	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_graminifolia	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_hamiltonii	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_nidiformis	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_paleacea	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_regia	????????	????????	????????	????????	????????	????????	????????	????????
Drosera_rotundifolia	TGAAAGTTT	TGAAAGTTT	TGAAAGTTT	TGAAAGTTT	TGAAAGTTT	TGAAAGTTT	TGAAAGTTT	TGAAAGTTT
Drosera_slackii	????????	????????	????????	????????	????????	????????	????????	????????
Drosophyllum_lusitanicum	GATTTTGATTT	GATTTTGATTT	GATTTTGATTT	GATTTTGATTT	GATTTTGATTT	GATTTTGATTT	GATTTTGATTT	GATTTTGATTT
Nepenthes_alata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_albomarginata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_boschiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_fusca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_glandulifera	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gracillima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_gymnamphora	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_hirsuta	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_insignis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_macfarlanei	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_maxima	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_mirabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_northiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_rafflesiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_reinwardtiana	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_sanguinea	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_spectabilis	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tentaculata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_tobiaca	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_truncata	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_ventricosa	-----	-----	-----	-----	-----	-----	-----	-----
Nepenthes_vieillardii	-----	-----	-----	-----	-----	-----	-----	-----
Triphyophyllum_peltatum	GATTCCT--	GATTCCT--	GATTCCT--	GATTCCT--	GATTCCT--	GATTCCT--	GATTCCT--	GATTCCT--

	2,690	2,700	2,710	2,720	2,730	2,740	2,750	2,760	2,770
Aldrovanda_vesiculosa	???	???	???	???	???	???	???	???	???
Ancistrocladus_abbreviatus	???	???	???	???	???	???	???	???	???
Ancistrocladus_barteri	???	???	???	???	???	???	???	???	???
Ancistrocladus_benomensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_cochichinensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_congolensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_grandiflorus	???	???	???	???	???	???	???	???	???
Ancistrocladus_guineensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_hamatus	???	???	???	???	???	???	???	???	???
Ancistrocladus_heyneanus	???	???	???	???	???	???	???	???	???
Ancistrocladus_korupensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_letestui	???	???	???	???	???	???	???	???	???
Ancistrocladus_robertsoniorum	???	???	???	???	???	???	???	???	???
Dionaea_muscipula	???	???	???	???	???	???	???	???	???
Dionophyllum_thollonii	???	???	???	???	???	???	???	???	???
Drosera_binata	???	???	???	???	???	???	???	???	???
Drosera_capensis	???	???	???	???	???	???	???	???	???
Drosera_dielisiana	???	???	???	???	???	???	???	???	???
Drosera_falconeri	???	???	???	???	???	???	???	???	???
Drosera_graminifolia	???	???	???	???	???	???	???	???	???
Drosera_hamiltonii	???	???	???	???	???	???	???	???	???
Drosera_nidiformis	???	???	???	???	???	???	???	???	???
Drosera_paleacea	???	???	???	???	???	???	???	???	???
Drosera_regia	???	???	???	???	???	???	???	???	???
Drosera_rotundifolia	???	???	???	???	???	???	???	???	???
Drosera_slackii	???	???	???	???	???	???	???	???	???
Drosophyllum_lusitanicum	???	???	???	???	???	???	???	???	???
Nepenthes_alata	???	???	???	???	???	???	???	???	???
Nepenthes_albomarginata	???	???	???	???	???	???	???	???	???
Nepenthes_boschiana	???	???	???	???	???	???	???	???	???
Nepenthes_fusca	???	???	???	???	???	???	???	???	???
Nepenthes_glandulifera	???	???	???	???	???	???	???	???	???
Nepenthes_gracillima	???	???	???	???	???	???	???	???	???
Nepenthes_gymnamphora	???	???	???	???	???	???	???	???	???
Nepenthes_hirsuta	???	???	???	???	???	???	???	???	???
Nepenthes_insignis	???	???	???	???	???	???	???	???	???
Nepenthes_macfarlanei	???	???	???	???	???	???	???	???	???
Nepenthes_maxima	???	???	???	???	???	???	???	???	???
Nepenthes_mirabilis	???	???	???	???	???	???	???	???	???
Nepenthes_northiana	???	???	???	???	???	???	???	???	???
Nepenthes_rafflesiana	???	???	???	???	???	???	???	???	???
Nepenthes_reinwardtiana	???	???	???	???	???	???	???	???	???
Nepenthes_sanguinea	???	???	???	???	???	???	???	???	???
Nepenthes_singalana	???	???	???	???	???	???	???	???	???
Nepenthes_spectabilis	???	???	???	???	???	???	???	???	???
Nepenthes_tentaculata	???	???	???	???	???	???	???	???	???
Nepenthes_tobiaca	???	???	???	???	???	???	???	???	???
Nepenthes_truncata	???	???	???	???	???	???	???	???	???
Nepenthes_ventricosa	???	???	???	???	???	???	???	???	???
Nepenthes_vieillardii	???	???	???	???	???	???	???	???	???
Triphyophyllum_peltatum	???	???	???	???	???	???	???	???	???

	2,780	2,790	2,800	2,810	2,820	2,830	2,840	2,850
Aldrovanda_vesiculosa	???	???	???	???	???	???	???	???
Ancistrocladus_abbreviatus	???	???	???	???	???	???	???	???
Ancistrocladus_barteri	???	???	???	???	???	???	???	???
Ancistrocladus_benomensis	???	???	???	???	???	???	???	???
Ancistrocladus_cochichinensis	???	???	???	???	???	???	???	???
Ancistrocladus_congolensis	???	???	???	???	???	???	???	???
Ancistrocladus_grandiflorus	???	???	???	???	???	???	???	???
Ancistrocladus_guineensis	???	???	???	???	???	???	???	???
Ancistrocladus_hamatus	???	???	???	???	???	???	???	???
Ancistrocladus_heyneanus	???	???	???	???	???	???	???	???
Ancistrocladus_korupensis	???	???	???	???	???	???	???	???
Ancistrocladus_letestui	???	???	???	???	???	???	???	???
Ancistrocladus_robertsonianorum	???	???	???	???	???	???	???	???
Dionaea_muscipula	???	???	???	???	???	???	???	???
Dionophyllum_thollonii	???	???	???	???	???	???	???	???
Drosera_binata	???	???	???	???	???	???	???	???
Drosera_capensis	???	???	???	???	???	???	???	???
Drosera_dielisiana	???	???	???	???	???	???	???	???
Drosera_falconeri	???	???	???	???	???	???	???	???
Drosera_graminifolia	???	???	???	???	???	???	???	???
Drosera_nidiformis	???	???	???	???	???	???	???	???
Drosera_paleacea	???	???	???	???	???	???	???	???
Drosera_regia	???	???	???	???	???	???	???	???
Drosera_rotundifolia	???	???	???	???	???	???	???	???
Drosera_slackii	???	???	???	???	???	???	???	???
Drosophyllum_lusitanicum	???	???	???	???	???	???	???	???
Nepenthes_alata	???	???	???	???	???	???	???	???
Nepenthes_albomarginata	???	???	???	???	???	???	???	???
Nepenthes_boschiana	???	???	???	???	???	???	???	???
Nepenthes_fusca	???	???	???	???	???	???	???	???
Nepenthes_glandulifera	???	???	???	???	???	???	???	???
Nepenthes_gracillima	???	???	???	???	???	???	???	???
Nepenthes_gymnamphora	???	???	???	???	???	???	???	???
Nepenthes_hirsuta	???	???	???	???	???	???	???	???
Nepenthes_ignis	???	???	???	???	???	???	???	???
Nepenthes_macfarlanei	???	???	???	???	???	???	???	???
Nepenthes_maxima	???	???	???	???	???	???	???	???
Nepenthes_mirabilis	???	???	???	???	???	???	???	???
Nepenthes_northiana	???	???	???	???	???	???	???	???
Nepenthes_rafflesiana	???	???	???	???	???	???	???	???
Nepenthes_reinwardtiana	???	???	???	???	???	???	???	???
Nepenthes_sanguinea	???	???	???	???	???	???	???	???
Nepenthes_singalana	???	???	???	???	???	???	???	???
Nepenthes_spectabilis	???	???	???	???	???	???	???	???
Nepenthes_tentaculata	???	???	???	???	???	???	???	???
Nepenthes_tobiaca	???	???	???	???	???	???	???	???
Nepenthes_truncata	???	???	???	???	???	???	???	???
Nepenthes_ventricosa	???	???	???	???	???	???	???	???
Nepenthes_vieillardii	???	???	???	???	???	???	???	???
Triphyophyllum_peltatum	???	???	???	???	???	???	???	???

	2,860	2,870	2,880	2,890	2,900	2,910	2,920	2,930	2,940
Aldrovanda_vesiculosa	???	???	???	???	???	???	???	???	???
Ancistrocladus_abbreviatus	???	???	???	???	???	???	???	???	???
Ancistrocladus_barteri	???	???	???	???	???	???	???	???	???
Ancistrocladus_benomensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_cochichinensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_congolensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_grandiflorus	???	???	???	???	???	???	???	???	???
Ancistrocladus_guineensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_hamatus	???	???	???	???	???	???	???	???	???
Ancistrocladus_heyneanus	???	???	???	???	???	???	???	???	???
Ancistrocladus_korupensis	???	???	???	???	???	???	???	???	???
Ancistrocladus_letestui	???	???	???	???	???	???	???	???	???
Ancistrocladus_robertsoniorum	???	???	???	???	???	???	???	???	???
Dionaea_muscipula	???	???	???	???	???	???	???	???	???
Dionophyllum_thollonii	???	???	???	???	???	???	???	???	???
Drosera_binata	???	???	???	???	???	???	???	???	???
Drosera_capensis	???	???	???	???	???	???	???	???	???
Drosera_dielisiana	???	???	???	???	???	???	???	???	???
Drosera_falconeri	???	???	???	???	???	???	???	???	???
Drosera_graminifolia	???	???	???	???	???	???	???	???	???
Drosera_nidiformis	???	???	???	???	???	???	???	???	???
Drosera_paleacea	???	???	???	???	???	???	???	???	???
Drosera_regia	???	???	???	???	???	???	???	???	???
Drosera_rotundifolia	???	???	???	???	???	???	???	???	???
Drosera_slackii	???	???	???	???	???	???	???	???	???
Drosophyllum_lusitanicum	???	???	???	???	???	???	???	???	???
Nepenthes_alata	???	???	???	???	???	???	???	???	???
Nepenthes_albomarginata	???	???	???	???	???	???	???	???	???
Nepenthes_boschiana	???	???	???	???	???	???	???	???	???
Nepenthes_fusca	???	???	???	???	???	???	???	???	???
Nepenthes_glandulifera	???	???	???	???	???	???	???	???	???
Nepenthes_gracillima	???	???	???	???	???	???	???	???	???
Nepenthes_gymnamphora	???	???	???	???	???	???	???	???	???
Nepenthes_hirsuta	???	???	???	???	???	???	???	???	???
Nepenthes_ignis	???	???	???	???	???	???	???	???	???
Nepenthes_macfarlanei	???	???	???	???	???	???	???	???	???
Nepenthes_maxima	???	???	???	???	???	???	???	???	???
Nepenthes_mirabilis	???	???	???	???	???	???	???	???	???
Nepenthes_northiana	???	???	???	???	???	???	???	???	???
Nepenthes_rafflesiana	???	???	???	???	???	???	???	???	???
Nepenthes_reinwardtiana	???	???	???	???	???	???	???	???	???
Nepenthes_sanguinea	???	???	???	???	???	???	???	???	???
Nepenthes_singalana	???	???	???	???	???	???	???	???	???
Nepenthes_spectabilis	???	???	???	???	???	???	???	???	???
Nepenthes_tentaculata	???	???	???	???	???	???	???	???	???
Nepenthes_tobiaca	???	???	???	???	???	???	???	???	???
Nepenthes_truncata	???	???	???	???	???	???	???	???	???
Nepenthes_ventricosa	???	???	???	???	???	???	???	???	???
Nepenthes_vieillardii	???	???	???	???	???	???	???	???	???
Triphyophyllum_peltatum	???	???	???	???	???	???	???	???	???

	3,030	3,040	3,050	3,060	3,070	3,075
Aldrovanda_vesiculosa	????????	????????	????????	????????	????????	????????
Ancistrocladus_abbreviatus	????????	????????	????????	????????	????????	????????
Ancistrocladus_barteri	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	ATTAATGGGACC	????????
Ancistrocladus_benomensis	GATCTTCCCA	GAGCAATGATA	CCATTTTATTTG	-----	ATTAATGGGACC	????????
Ancistrocladus_cochichinensis	????????	????????	????????	????????	????????	????????
Ancistrocladus_congolensis	????????	????????	????????	????????	????????	????????
Ancistrocladus_grandiflorus	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	ATTAATGGGACC	????????
Ancistrocladus_guineensis	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	ATTAATGGGACC	????????
Ancistrocladus_hamatus	????????	????????	????????	????????	????????	????????
Ancistrocladus_heyneanus	????????	????????	????????	????????	????????	????????
Ancistrocladus_korupensis	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	ATTAATGGGACC	????????
Ancistrocladus_letestui	????????	????????	????????	????????	????????	????????
Ancistrocladus_robertsoniorum	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	ATTAATGGGACC	????????
Dionaea_muscipula	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	ATTAATGGGACC	????????
Dioncophyllum_thollonii	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	ATTAATGGGACC	????????
Drosera_binata	????????	????????	????????	????????	????????	????????
Drosera_capensis	????????	????????	????????	????????	????????	????????
Drosera_dielisiana	????????	????????	????????	????????	????????	????????
Drosera_falconeri	????????	????????	????????	????????	????????	????????
Drosera_graminifolia	????????	????????	????????	????????	????????	????????
Drosera_hamiltonii	????????	????????	????????	????????	????????	????????
Drosera_nidiformis	????????	????????	????????	????????	????????	????????
Drosera_paleacea	????????	????????	????????	????????	????????	????????
Drosera_regia	????????	????????	????????	????????	????????	????????
Drosera_rotundifolia	GTTCTTCCCA	GAGCAATGATA	CAC-----	TATTT	-GCTTGTCTGATGGGACC	????????
Drosera_slackii	????????	????????	????????	????????	????????	????????
Drosophyllum_lusitanicum	????????	????????	????????	????????	????????	????????
Nepenthes_alata	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_albomarginata	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_boschiana	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_fusca	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_glandulifera	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_gracillima	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_gymnamphora	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_hirsuta	????????	????????	????????	????????	????????	????????
Nepenthes_ignis	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_macfarlanei	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_maxima	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_mirabilis	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_northiana	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_rafflesiana	????????	????????	????????	????????	????????	????????
Nepenthes_reinwardtiana	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_sanguinea	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_singalana	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_spectabilis	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_tentaculata	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_tobiaca	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_truncata	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_ventricosa	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Nepenthes_vieillardii	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????
Triphyophyllum_peltatum	GTTCTTCCCA	GAGCAATGATA	CCATTTTATTT	-G-----	CTGATGGAAACC	????????

A.4

Voucher information for collected specimens utilized in chapter 2

Voucher information includes the names of taxa from which class I chitinases were amplified for this study, details of the tissue collection, and herbaria where the vouchers have been deposited. Greenhouse-grown specimens cultivated at the Botanical Gardens of the University of California, Berkeley (UCBG), Missouri Botanical Garden (MO) or Botanischer Garten der Universität Würzburg (BGW). Voucher specimens are deposited in the following herbaria: Missouri Botanical Garden = MO, The University and Jepson Herbaria of the University of California, Berkeley = UC, Universität Würzburg = UW.

Taxon; Voucher specimen or living collection number; DNA collection number; Collection locale; Herbarium.

Ancistrocladus grandiflorus; R.E. Gereau 5557; TR122; MO cultivated; MO.

Ancistrocladus robertsonianorum; K.M. Meyer 277; TR124; MO cultivated; MO.

Dionaea muscipula; 2009.0254; TR186; UCBG cultivated; UC.

Drosera binata; 2001.0104; TR03; UCBG cultivated; UC.

Drosera capensis; 69.0172; TR05; UCBG cultivated; UC.

Nepenthes maxima; 76.1342; TR22; UCBG cultivated; UC.

Nepenthes mirabilis; 95.1378; TR23; UCBG cultivated; UC.

Triphyophyllum peltatum; UW plant culture; TR121; BGW cultivated.

A.5

Multiple sequence alignment of translated CDS for angiosperm class I chitinases

The following four pages consist of a multiple sequence alignment of translated CDS for angiosperm class I and IV chitinase homologs identified. Chitinase sequences are grouped based on phylogenetic placement in figure 2.1. Colors highlight the following chitinase domains: yellow, signal peptide; green, cysteine-rich domain; red, proline-rich hinge; orange, catalytic domain; cyan, CTE. The seven active sites (I-VII) of the catalytic domain are indicated above the alignment, which correspond to regions described by Garcia-Casado et al. (1998), Bishop et al. (2000), and Tiffen (2004). Poorly aligned regions (1-93, 116-148, 390-440) including the proline-rich hinge, N- and C- termini were removed for phylogenetic analyses (figs. 2.1 and 2.2). GAP = - and MISSING = ?

1 10 20 30 40 50 60 70 80 90 100 110

NmirChitl-1
NmirChitl-2
NkChitl-1
NmaxChitl-1
NmaxChitl-2
NkChitl-2
TpChitl-2
TpChitl-1
AgChitl-1
ArChitl-1
LjChitl-1
PtChitl-1
PtChitl-2
PtChitl-4
PtChitl-3
PtChitl-5
AtChitl-1
DrChitl-1
GmChitl-1
GmChitl-2
MkChitl-1
VvChitl-4
DmChitl-1
NmirChitl-3
NkChitl-3
NkChitl-4
TpChitl-3
AgChitl-2
AgChitl-3
ArChitl-2
DrChitl-3
DrChitl-2
DcChitl-1
DbChitl-1
DbChitl-2
DsChitl-1
DmChitl-2
BdChitl-1
BdChitl-2
BdChitl-3
BdChitl-4
OsChitl-1
OsChitl-4
OsChitl-3
SbChitl-2
ZmChitl-2
OsChitl-2
SbChitl-1
ZmChitl-1
AtChitlV
NaChitlV
OsChitlV
SbChitlV
VvChitlV

MEIASAKIFFGLSLLG-LLALGSAEQCGSOAGGAVCPGGLCCSOYGMCGTTDDYCGAGCQSQCS
MEIASAKIFFGLSLLG-VLALGSAEQCGSOAGGAAPEGGGCCSOFGHCCTDDYCEAGCQSQCS
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MKIWFVSVF SLLL SLLFLVGSAEQCGROAGGALCPGGQCCSOFGWCGTNDAYCSKGCQSQCG
MKIWAFTV SLLL SLLGGSAEQCGSOAGGAVCPGGLCCSOFGWCGTNDYCGNGCQSQCG
MKMWFTVF SLLL SLLGGSAEQCGSOAGGALCPGGQCCSOFGWCGTNDYCGNGCQSQCG
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MSWAFVFF SLLFLSLVSRGSAEQCGROAGDALCPGGICGSKYGCSTSEYCGEGCQSQCG
MPPOKENHRTLNKMTNLFLLF SLLSLSAEQCGROAGGALCPNGLCCSEFFGWCGTTEPYCKQPGCQSQCG
MKNMKLCSVMLCLSLAFLLGATAEQCGTOAGGALCPNRLCCSKFVGCDDTDSYCGEGCQSQCG
MLCLLAFLLGAAQNCGTQVGGVCPNGLCCSOYGMCGTTEAHCGRGCQSQCT
MKLQMGSTT--KL-SCLLCLLAFLLGSKAQCGROANGAVCANRLCCSOFGYCGTADYCGAGCQSQCT
MKIWGLRFLPEMLLAIGGAFAEQCGROAGGALCSGGL-----
MNAFCFCFHAQKMRNHKXSTMRGVVLLLNLPFLSAFOCGOAGGALCHSGLCCSOWGCGTTSYCYGNGCQSQCG
MNAFCFCFHAQKMRNHKXSTMRGVVLLLNLPFLSAFOCGOAGGALCHSGLCCSOWGCGTTSYCYGNGCQSQCG
KDCQSQCG
QSQCG
QDCQSQCG
MRELA VVLAAMLAAMAMSVRGEQCGSOAGGATCPNCLCCSRFGWCGSTPDYCLTGCQSQCS
MVHFFPAA MRELA VVLAAMLAAMAMSVRGEQCGSOAGGATCPNCLCCSRFGWCGSTPDYCLTGCQSQCS
MRAIVVAMLVAAALLAVSARAQCGSOAGGATCPNCLCCSRFGWCGSTPDYCLTGCQSQCS
MKSAAVV-AMLAAILLAVFARTEQCGSOAGGATCPNCLCCSRFGWCGSTPDYCLTGCQSQCS
MRALAVVAVATAFLAAVHAFOCGSOAGGAVCPNCLCCSOFGWCGTSDYCGAGCQSQCS
MRSHRFLTSLQSSKQANSTLL-CTATMRALAVVV-ATAFVVAVRGEQCGSOAGGALCPNCLCCSOFGWCGTSDYCYGNGCQSQCS
MRALAVV--AMAVVAVRGEQCGSOAGGALCPNCLCCSOFGWCGTSDYCGAGCQSQCS
MTMRALLAVATMVAVFLSMSSTHAQCGTOAAGALCPNCCOSMYGWCGTSDYCYGNGCQSQCT
MSTPRAA-ALAMVAMLAALFPM SARAQCGTOAGGALCPNCCOSMYGWCGTSDYCYGNGCQSQCF
MSTPRAA-SLAKKALVALAVLAAA-LATAARAFOCGOAGGALCPNCLCCSRFGWCGTSDYCYGNGCQSQCS
MWTGQCN EATCGDFAPATTESMCKRCQSAPYPAAKTQSRSTSGGATPPRQATTAVALVLAVALASGDFQCGSOAGGATURDCLCCSOFGFCGDTSAVCAGCQSQCT
MTESMYVYKIPAVRALPGSKDORMSTPPGAP SHATTAVALCVLAALAVTVA SGOOCGOAGGATCRDCLCCSRFGWCGTSDYCYGNGCQSQCT
MALSAEINLLTVAFLAGLIPNLVSGONCG-----CAANLCCSOFGYCGTDDAYCGVGRSGPC
MAFTKISIVL LLLCLGFFSETVKSONCG-----CAPNLCCSOFGYCGTDDAYCGVGRSGPC
MARRLSLLAVVLAAMVAASATAAQSCG-----CASDCCSKWGFCTGDYCGPGGOAGPC
MENAPRILTWALGALLCAGAGPAAAQNCG-----COPGYCCSOYGYCGKGDYCGKGRSGPC
MGFC CCI VVPHKDLGFKYKPYTKVSTLSSHFSHSSLDWAIKVAILLVGLAGALPAFVLAQNCG-----CDAGLCCSOWGYCGTGDYCGVGRSGPC

360 370 380 390 400 410 420 430 440

VI

VII

- NmirChiti-1
- NmirChiti-2
- NkChiti-1
- NmaxChiti-1
- NmaxChiti-2
- NkChiti-2
- TpChiti-1
- TpChiti-2
- ArChiti-1
- ArChiti-2
- LjChiti-1
- PtChiti-1
- PtChiti-2
- PtChiti-3
- PtChiti-4
- PtChiti-5
- PtChiti-6
- DrChiti-1
- DrChiti-2
- GmChiti-1
- GmChiti-2
- MfChiti-1
- VvChiti-1
- VvChiti-2
- DmChiti-1
- NmirChiti-3
- NkChiti-3
- NkChiti-4
- TpChiti-3
- TpChiti-4
- AgChiti-2
- AgChiti-3
- ArChiti-2
- ArChiti-3
- DrChiti-2
- DrChiti-3
- DcChiti-1
- DcChiti-2
- DbChiti-1
- DbChiti-2
- DsChiti-1
- DsChiti-2
- DmChiti-2
- BdChiti-1
- BdChiti-2
- BdChiti-3
- BdChiti-4
- OsChiti-1
- OsChiti-2
- OsChiti-3
- OsChiti-4
- OsChiti-5
- ZmChiti-1
- ZmChiti-2
- ZmChiti-3
- ZmChiti-4
- ZmChiti-5
- AtChitiV
- NaChitiV
- OsChitiV
- SbChitiV
- VvChitiV

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

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INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~

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INGGVECGHGQDARV-ADRI~~GFYKRY~~CDLILGVGYGNLDDCYNORPFGN~~GILLWATE~~