國立交通大學

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碩 士 論 文

探究造癭昆蟲基因分子演化關係及蟲癭組織蛋白質體組成

-以樟科楨楠屬植物及癭蚋科昆蟲為例

Molecular Phylogenetic Relationships of Cecidomyiidae Gall Midges

and Proteomics of Galls from *Machilus* Hosts

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摘　　要

本論文主要分兩方面探討台灣楨楠屬植物上之雙翅目癭蚋科蟲癭的生長情況。一由基因序列演化分析探討雙翅目癭蚋科蟲癭彼此間的親源遠近關係，再利用二維電泳探究蟲癭組織與植物組織當中蛋白質成分的差異，以期瞭解蟲癭的發生機制。並綜合討論癭蚋分子演化關係、蟲癭型態發生、與癭蚋外型解剖間相互的關係。本研究取台灣八種楨楠上的九型蟲癭，共四十種樣本做癭蚋基因演化關係。而蟲癭組織蛋白質分析取三種楨楠、兩型蟲癭，共六種樣本作蛋白質二維電泳及定序。由造癭昆蟲基因演化分析得知，造出同型蟲癭的癭蚋，為分類學上較親近的種。種的親緣遠近關係與寄主植物的種類較無關連。而造莖部癭的癭蚋較造葉部癭的癭蚋早演化出來。而癭蚋幼蟲解剖分析支持前述推論。植物分類學上，莖部癭所具有之型態特徵亦被認為是較早出現的形態。由蟲癭組織蛋白質體二維電泳分析得知，不同型蟲癭彼此間蛋白質差異不大，但蟲癭外型與蛋白質相關。同型蟲癭在不同楨楠屬植物上的蛋白質差異較前述為大，可能因植物種類不同，對相似化學物質的反應不同所致。蟲癭組織當中RuBisCO蛋白質表現量較正常植物組織大幅下降，進而推測蟲癭組織已喪失光合作用的功能。

Molecular Phylogenetic Relationships of Cecidomyiidae Gall Midges and Proteomics of Galls from Machilus Hosts

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Abstract

Cecidology is the study of plant galls, where insects and other organisms breed their offsprings. Cecidology crosses multiple areas such as ecology, insect taxonomy, botanical taxonomy, botanic pathology, parasitology, and it's a special research subject. Little is known regarding the interaction between gall insects and their host at molecular level.

In Taiwan, *Machilus* (Lauraceae) plants have the widest variety of gall shapes among all plant species. Cecidomyiidae (Dipteria) is the major gall inducing insects on *Machilus*. To understand the relationship between gall insects and their hosts, we first identified different species of gall insect through insect genomics and the responses of the host were studied through plant proteomics. In insect genomics, we used COI gene to classify 40 unknown species collected from 9 different types of galls on 8 *Machilus* species. These cecidomyiidae gall inducing midges could be classified into 7 tribes, 11 genus, and 15 species. Our data indicate that insects which make same type of galls are closer species. The anatomical evidence and ecological theories also supports genomic evidence.

In plant proteomics, we found that galls were benignancy tissue growth of plant. Because RuBisCO protein expression in gall tissues was largely decreased, galls might be the storage organisms of plants. Combine genomic and proteomic outcomes, we suggest that same or closer insect species secretes same chemicals to induce same shape of galls, and it has nothing to do with host plants within Machilus genus. Some proteins might also affect the forming of different gall shapes as significant protein variations were observed between gall and normal plant tissues.

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韋汝

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Abbreviations and Symbols

PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic acid
NHRI	National Health Research Institute
COI	Cytochrome Oxidase I
UV	Ultraviolate
NCBI	National Center for Biotechnology Information
TFRI	Taiwan forest Research Institute
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
UPGMA	Unweighted Pair Group Method with Arithmatic Mean
NJ	Neighbor Joining

1. Introduction

1.1 The importance of correct taxonomy DNA barcode

Accurate taxonomy plays an important role in ecological, evolutionary interpretation, and many other practical applications. Correct identification of species is an essential part of taxonomy due to the fact that traditional morphology-anatomy-based classification methods have some limitations (Edward, 2006; Gdofray, C., 2004; Samper, C., 2004). First, it might overlook some morphologically similar species, which are familiar to many taxa (M. Alex Smith et al, 2006; Hebert P.D.N. et al, 2004; Jarman et al, *J,* 2000). Second, it sometimes only works in certain life stages or genders and demands high level of expertise which made classification a long and difficult task (Hebert, P.D.N. et al, 2003). With those limitations, and species around the world are actually disappearing faster than biologists can identify them, there's a press need for rapid, accurate methods of classifying life (Meter CP, Paul G, 2005).

DNA barcode has recently been proposed as a tool to facilitate species identification and discovery (Hebert, P.D.N. et al, 2003; Blaxter M, 2003). The theory base of DNA barcode is that a short and standardized segment of genome can be regard as a "biological barcode" which can be used in species identification (Marshall, E., 2005). It could be a helpful tool of species recognition because sequence divergences among individuals in the same species are normally much lower than that between related species (Avise, J.C. & Walker D. 1999; Hebert P.D.N. et al, 2003; Moore, W.S, 1995). In prior researches, DNA barcodes has been used to identify species among parasitoid flies (M. Alex Smith et al, 2006), skipper butterflies (Hebert P.D.N. et al, 2004), Lepidoterans (Hebert, P.D.N. et al, 2003), birds (Hebert P.D.N. et al, 2004), and even flowering plants (Kress W. J. et al, 2005).

In early phylogenetic studies, mitochondria 12S, 16S, and 28S subunit genes have been used (Hwang J.S. et al, 1999; Dorchin et al, 2004; Mollaret et al, 2000), but scientists found that the prevalence of insertions and deletions (indels) of these genes could increase the complexity of sequence alignment and led to constraint on broad taxonomic analysis (Doyle et al, 2000). By contrast, the cytochrome c oxidase I gene segments (COI) has two significant advantages: First, the universal primers for COI are very robust which could recover the 5' end of most representative species in animal phyla (Folmer et al, 1994; Zhang et al, 1997). Second, because the third-position nucleotides have high rates of substitution which leads to greater molecular evolution rate than 12s or 16s rDNA, and it indicates that COI could possess a better range of phylogenetic signals (Knowlton et al, 1998). Therefore COI has replaced those genes and been used for animal classifications recently [5, 8, 11-13]. Among Diptera insects, COI is wildly used for classification and identify new species (Jin-Sik Bae et al, 2001; James M. Cook et al, 2002; N. Uechi et al, 2003; J. Yukawa et al, 2003; Makoto Tokuda et al, 2004).

The effectiveness and accuracy of COI DNA barcodes has been proved to be as high as 97.9% by testing the 521 recognized species in three different families of Lepidoptera-Hesperiidae (skipper butterflies), Sphingidae (sphinx moths), and Saturniidae (wild silk moths) (Mehrdad H. et al, 2006).

1.2 About gall-inducing insects

Galls are the abnormal growth of plant tissues which can be induced by infections of nematodes, bacteria, fungi, virus, or insect stimulation. Insect galls can be found on angiosperm, gymnosperm, pteridophyte, lichen, algae, but angiosperm are the major gall plants, which has the ratio of 90% and up to all kinds of galls. Galls can be found on any plant species and organisms but 80 % can be found on leaves (Mani, 1964).

The oldest gall insect might be evolved from saprophytic insects during Devonian, but most of the gall insects nowadays are evolved from plant-eating and carnivorous insects (Roskam, 1992).

Insect galls develop under the influence of gall-inducing insects. Insect galls are usually induced by the chemicals injected by the larvae or the adults in the plants, either including mechanical damage or not. After the galls are formed, the larvae develop inside until fully grown, at which time they leave, sometimes as adults. In order to form galls, the insects must seize the time when plant cell division occurs at a high speed, the growing season, usually spring in temperate climates, but which can be extended in tropical latitudes. Also, the specific places where plant cell division occurs are needed to induce galls, that is, the meristems. Although insect galls can be found on a variety of parts of the plant, such as the leaves, stalks, branches, buds, roots or even flowers and fruits, gall-inducing insects are usually species-specific and sometimes tissue-specific on the plants they gall. Some insects induce galls on plants similar to each other, frequently within genera or family.

Gall-inducing insects include gall wasps, gall midges, aphids, and psyllids.

In former researches, we know that insect has high specificity of their host plants and organisms that they only make galls on the same host or with in some specific and related species (Dreger-Jauffert & Shorthouse, 1992). Among all the gall-inducing insects, the family Cecidomyiidae (Diptera) is believed to be one of the major groups of gall inducers and most gall midges are highly host specific (Harris, K.M. 1994).

There are over 15,000 species of known gall insects and distributed in 6 orders. Which include: Diptera, Hymenoptera, Lepidoptera, Coleoptera, Hemiptera, Thysanoptera (Meyer, 1987; Williams, 1994).

In Taiwan, Cecidomyiidae (Diptera) is the major gall-inducing insects of the genus *Machilus* (Lauraceae) plant, which has the widest varieties of gall forms among all the galled plants. Cecidomyiidae midges are very fragile small insects usually only 2-3 mm. in length and many are less than 1 mm long. They are characterized by hairy wings, unusual in the Order Diptera, and have long antennae. Unlike some gall-inducing insects of other plant species, most of Cecidomyiidae midges are unknown species and it's hard to classify them by their appearance. In order to solve this problem, we adopt the molecular classification method, DNA barcode.

1.3 About plant galls

Galls are the abnormal growth of plant tissues which can be induced by infections of nematodes, bacteria, fungi, virus, or insect stimulation. Insect galls can be found on angiosperm, gymnosperm, pteridophyte, lichen, algae, but angiosperm are the major gall plants, which has the ratio of 90% and up to all kinds of galls. Galls can be found on any plant species and organisms but 80 % can be found on leaves (Mani, 1964).

According to the level of diversification, galls are classified in to organoid gall and histoid gall (Kuster, 1911). Organoid galls are the normal changes of plant organs which gall-inducing insects induced, and the original plant organs can still be distinguished. Histoid galls are the abnormal growth or proliferation of plant tissues, which can be divided further into kataplasmatic gall and prosoplasmatic gall.

Normally, galls are classified according to their shape, conformation complexity, and positions on plants. There are many types: filz gall, pit gall, blister gall, pouch gall, roll gall, fold gall, covering gall, mark gall, bud gall, and rosette gall (shorthouse, J.D, 1992). There are also other classification method which according to the forms of insect locules. A single gall with only one insect inside is monolocular gall; many monolocular galls aggregate together are called gregarious gall; If gall-inducing insect laid many eggs at the same position of plant organ, and developed one gall with several locules, it's called multilocular gall. (Csoka, 1997)

The mechanisms of insect gall development are not well studied, and they're still unclear. The possible mechanisms include: 1.Injection of saliva while insects eat plant tissues, there might be some inducer factors in insect saliva. 2.Injection of gall-inducing factors while insects lay their eggs. 3.Gall-inducing factors which exists in insects or larvae's feces. (Higton, Mabberly, 1994). The most accepted mechanism theory is injection of gall-inducing factors while insects spawning. But gall-inducing factors are still unknown. According to past researches, the possible gall-inducing factor might be some plant growth factors, which includes indole acetic acid (IAA), cytokinins, auxins, and zeatins (Cornell, 1983; Abrhamson, 1997). Other possible gall-inducing-factors might include amino acid (Schaller, 1969), proteins (Higton, Mabberly, 1994).

1.4 About *Machilus* in Taiwan

The Lauraceae or Laurel family comprises a group of flowering plants included in the order Laurales. The family contains about 55 genera and over 2000 (perhaps as many as 4000) species world-wide, mostly from warm or tropical regions, especially Southeast Asia and Brazil. Most are aromatic evergreen trees or shrubs. There are about 20 genera and over 400 species in China, and about 14 genus 60 species in Taiwan.

The leaves are simple, without stipules, and usually alternate. The androecium most frequently comprises 4 whorls of 3 stamens each, although the inner whorls are often sterile. The flowers are actinomorphic, usually bisexual, and possess a perianth of six, basally connate sepallike segments. The anthers dehisce by means of commonly 4, upwardly opening flaps. The filaments of the inner whorl usually have a pair of enlarged glandular appendages near the base. The fruit is a berry or a drupe, often surrounded basally by the short, persistent perianth cup. The single simple pistil has a usually superior ovary with a single pendulous ovule in a solitary locule. Unlike other Magnoliidae, the endosperm is completely absorbed by the embryo in Lauraceae. (wikipedia: Lauceae)

Machilus is a genus of about 150 species of evergreen trees belonging to the laurel family, Lauraceae. It's classification is Kingdom Plantae, Phylum Magnoliophyta, Class Magnoliopsida, Order Laurales, Family Lauraceae.

They are medium-size trees, 15-30 m tall at maturity. The leaves are simple, lanceolate to broad lanceolate, varying with species from 5-30 cm long and 2-12 cm broad, and arranged spirally or alternately on the stems. The flowers are in short panicles, with six small greenish-yellow perianth segments 3-6 mm long, nine stamens and an ovary with a single embryo. The fruit is an oval or pear-shaped drupe, with a fleshy outer covering surrounding the single seed; size is very variable between the species. (wikipedia: Machilus)

The major distribution of *Machilus* is in tropical and subtropical zones, especially in East Asia, about 100 species. None of the species is very tolerant of severe winter cold.

There are eight *Machilus* species found in Taiwan. There are two varieties each were classified for M. japonica Sieb & Zucc. and M. zuihoensis Hay. The two varieties of M. zuihoensis Hay. are M. zuihoensis var. zuihoensis (M. zuihoensis, the acronym is MZ in sample classification table) and M. zuihoensis var. mushaensis (M. mushaensis, MM). The two varieties of M. japonica Sieb & Zucc. are M. japonica var. japonica (M. japonica,) and M. japonica var. kusanoi Hay. (M. kusanoi, MJK). Other species are M. philippinensis Merr. (MP), M. konishii Hay. (MK), M. obovatifolia Hay. (MO), and M. thunbergii (MT). Among these eight species, MZ, MK, MO, and MK are the endemic species of Taiwan. (Miao et al, 2007)

The distributions of some *Machilus* species within Taiwan are restricted, but others are found throughout large parts of the island. *MT MJ* and *MZ* are widespread from subtropical to temperate zones; *MJK* is also widely distributed but mainly in the low lands near rivers. *MK* is restricted to the subtropical zone in central and southern parts of Taiwan west of the Central

Mountain Range, and *MP* is only found in the subtropical southern part west of the Central Mountain Range. *MO* is only found on the tropical Hengchun Peninsula at the southern tip of the island. . These species have adapted to different edaphic and environmental conditions. (Miao et al, 2007)

1.5 Previous researches and our motivation

There are several researches about gall and plant physiology. For example, some scientists have tested the nitrogen concentration in plant primary metabolites, because nitrogen is usually regarded as the index of whether host plant could provide sufficient nutrition or not. But the outcomes are different among different researches. Hartley (1998) discovered that the nitrogen concentration is higher in *Dasineuravicia* galls, but not in other Diptera insect galls (*Rhopalomyia sp*.). Other researches revealed that the nitrogen concentrations are even higher in plant tissues than in gall tissues made by gall wasp, gall fly (Hartly, 1998) and some gall midge (Brewer et al, 1987).

Some researches indicated that there are obvious high concentration of starch, soluble carbohydrate, lipid and proteins in gall tissues. (Shannon 1980; Bronner, 1992; De Bruyn et al, 1998) But there are other researches indicated the opposite situations. (Anderson and mizell, 1987; Conell, 1983, Hartley, 1992, 1998; Yang 1998)

Most of previous researches in Taiwan about galls, focus on the description and comparison of newly discovered galls on plants. (Yang, 1984; Tao 1991; Yang and Tung, 1998). Some researches described more detailed insect life history and related information (Yang, 1996; Tung, 1998, Su 2002). There are also some researches about gall forms and tissues (Su, 2002; Tung, 1997; Liang, 1999; Weng, 2003; Chen, 2004). Other research about plant physiology and ecology includes Yang (1998) discuss the photo pigment and protein complex; Liao 2003 discussed the nutrition adaptation of gall-insects.

There are more and more researches about galls in Taiwan. Scientists put more attentions and interests on these abundant galls. In our research, we use 2D electrophoresis technique to determine the protein differences between plant and gall tissues, in order to understand the protein changes. And we can provide further information of gall physiology.

We also use DNA barcode to determine gall midge phylogeny, combined with plant gall proteomics and larvae anatomy, in order to contribute some information to Cecidology.

2 .Materials and Methods

2.1 Insect gene molecular evolution

2.1.1 Gall midge sample collection

These samples was collected by Dr. Tung, Mr. Hsu and I. Total 9 morphospecies of galls were collected together with leaves and stems from 8 species of *Machilus* in Taiwan October 2004 through January 2005. Among these 9 morphospecies of galls, 3 are stem galls and 6 are leaf galls. We gave each plant species and galls a number. Since all midge larvae inside these galls are unknown species, we named these larvae according to plant names, gall types, and serial numbers. Detailed information is shown in Fig. 1 and Table 1.

Galls along with leaves and stems were put in zipped bags in 4℃ refrigerator. Larvae or pupae were picked from galls in two days after collection and preserved in 99.5% alcohol in 4 ℃ for DNA extraction. Every larva or pupae were picked over under dissecting binocular microscope. Larvae and pupae which were distinguishable or suspected parasitized were excluded in order to avoid contamination with internal or external parasitoid larvae in DNA analysis.

The way we treated parasitized larvae:

Cecidomyiidae midge larvae are easily be parasitized by bees or other organisms such as fungi, bacteria…etc. When parasites were inside the larvae, we abandoned these larvae. When parasites were outside the larvae, we picked parasites out from midge larvae. Then we put these once-parasitized larvae into another container, separated from those un-parasitized larvae in order to avoid contamination. We would use these once-parasitized larvae only when we ran out of un-parasitized larvae. In our experiment, we didn't use these once-parasitized larvae.

2.1.2 DNA extraction

Due to the various body sizes and weights of larvae, an average of total weight 5 mg individuals from respective types of galls and host plants were used for DNA analysis. Detailed data for speciment are shown in Table 1 and Table 2.

Total DNA were extracted from the whole body with QIAamp DNA Mini Kit (Qiagen) according to the steps in manufacturer's specification. A ca. 430 bp long fragment of the 12S small ribosomal subunit was PCR-amplified using the primers SR-J-14199 (50-TAC TAT GTT ACG ACT TAT-30) and SR-N-14594 (50-AAA CTA GGA TTA GAT ACC C-30) (Kambhampati and Smith, 1995). Another region of cytochrome oxidase subunit I (COI) gene of mitochondria was amplified by using the following primer pair : forward, 5'-GGA TCA CCT GAT ATA GCA TTC CC-3' (COIS) and reverse, 5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3' (COIA) (Funk, 1995). All PCR mixes had a total volume of 100 μl and contained 0.1mM dNTPs, 2 μM of each primer, 5-10 μl genomic DNA , one unit of *Taq*DNA polymerase (Protaq), 10 μl PCR buffer comes with TaqDNA polymerase (Protaq), and add ddH_2O to 100 μl. The thermocycling profile consisted of initial step of 5 min at 92℃, followed by 30 cycles of 1 min at 92℃, 1 min at 52℃, and 1 min at 72 ℃, with the final step of 5 min at 72℃. PCR products were electrophoresed in 2.0% TAE agarose gels along with 100bp DNA markers (violet), stained with ethidium bromide, and visualized under UV light. In some cases, the DNA band in agarose gel needs to be purified by GFX PCR DNA and gel band purification kit (Amersham Biosiciences) according to the manufacturer's instruction. The purified DNA is amplified the same way aforementioned. PCR products of each sample were sequenced then.

2.1.3 DNA analysis

All DNA sequence data were uploaded to SeqWeb along with 3 out-group sequences choose from NCBI. These 3 out-group sequences are also in the order Dipteria, but in different families with our sample midges. SeqWeb is provided by NHRI and it's also the internet surface of Winkinson Package.

Two analysis methods were used in our study, PileUp and Evolution.

Pileup function creates multiple alignments of several sequences. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a *cluster* of two aligned sequences. This cluster can then be aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences can be aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments that include increasingly dissimilar sequences and clusters, until all sequences have been included in the final pairwise alignment. Before alignment, the sequences are first clustered by similarity to produce a *dendrogram*, or tree representation of clustering relationships. It is this dendrogram that directs the order of the subsequent pairwise alignments.

In our analysis, the gap creation penalty is 5 and extension penalty is 1.

Evolution function investigates the relationships within a group of sequences. DNA sequences were analyzed by neighbor-joining method. (Saiton N, 1987 and UPGMA method. The arithmetic average of evolutionary distance was computed by Kimura 2-parameter. (Kimura, 1980). several sequences which presumed to be in the same family were used as out groups from NCBI.

Neighbor-Joining

This method is designed to find an approximation to the minimum evolution tree for a set of aligned sequences, using less computer time than the full algorithm for determining a minimum evolution tree. It works best when the distances are additive.

The neighbor-joining method clusters the sequences in a pairwise fashion. However, instead of picking the next pair to cluster by looking for the smallest distance in the distance matrix, this method seeks to form pairs that minimize the sum of the branch lengths for the entire tree. Therefore at each round of clustering, all possible pairs of entries are considered one at a time and the sum of the branch lengths for the resulting tree is calculated. The pairing that results in the smallest sum is the one that will be used to form the new cluster. This new cluster replaces its two constituent entries in the distance matrix (reducing the dimension of the distance matrix by one), and distances are calculated between the new cluster and the remaining entries in the distance matrix. The process continues until only two entries remain. The resulting tree is an unrooted tree. Because this method attempts to build an additive tree from the data, negative branch lengths may result if the distance data are not exactly additive

UPGMA

This method (Sneath and Sokal, *Numerical Taxonomy*, Freeman, San Francisco (1973)) can be used to estimate a species tree or gene tree when the expected rate of gene substitution is constant and the distance measure is linear with evolutionary time (for example, distance is measured as amino acid substitutions). The distances *must* be ultrametric to obtain a correct tree using this method.

The two sequences that have the smallest distance in the distance matrix are combined to form a cluster. That cluster replaces the original sequence pair as a single entry in the distance matrix (reducing the dimension of the matrix by one), and distances between the cluster and the other entries are calculated. The entries in the new matrix that have the smallest distance are combined to form a new cluster, and the process continues until only a single cluster remains. The resulting tree is a rooted tree.

Instead of using a simple average, the UPGMA method calculates the distances between a new cluster and the other entries in the distance matrix based on the total number of sequences in the cluster. If the new cluster *C* was formed by combining two clusters *a* and *b*, cluster *a* representing $N_{(a)}$ total sequences and cluster *b* representing $N_{(b)}$ total sequences, the distance between the new cluster *C* and another entry *k* is:

$$\text{distance}(k,C) = [\ \text{distance}(k,a) * N_{(a)} + \text{distance}(k,b) * N_{(b)}\ ] / (N_{(a)} + N_{(b)})$$

Kimura Two-Parameter Distance

This method applies only to nucleic acids and takes into consideration the fact that transition substitutions (purine-purine or pyrimidine-pyrimidine) often occur much more frequently than transversion substitutions (purine-pyrimidine). Gap positions and ambiguous symbols other than R (purine) and Y (pyrimidine) are not scored.

P = transitions / positions_scored

Q = transversions / positions_scored

$\text{distance} = -{}^{(1)}/_{(2)} \ln[\ (1 - 2P - Q) * \text{sqrt}(1 - 2Q)\ ]$

M. Kimura, J. Mol. Evol. **16**; 111-120 (1980).

This method gives better distance estimates than the Jukes-Cantor method when the rates of transitional and transversional substitutions are different. However, when the substitution pattern is more complex than this, this method underestimates the true distance for distantly related sequences.

SeqWeb http://v8803.nhri.org.tw:8003/mgr.shtml

NCBI http://www.ncbi.nlm.nih.gov/

2.2 Plant proteomics

The following plant proteomic experiments ware done by Hung-Pin Chen, my laboratory colleague. I collected and arranged his experimental data, and discuss these data together with my experimental data.

2.2.1 Sample collection:

Both plant galls and *Machilus* leaves are collected from Taiwan Fu-Shan Research Station (TFRI). Plant galls are commonly found on *Machilus zuihoensis var. mushaensis*, *Machilus zuihoensis var. zuihoensis*, *Machilus thunbergii*, *Machilus japonica, Machilus japonica kusanoi, Machilus philippinense, and Hamamelidaceae* in TFRI. There are also several different types of galls on each plant species as gall midge collection table shows [Table 1]. Among all types of galls and plant species, the most abundant galls are bell galls and mice galls on *Machilus thunbergii*, *Machilus zuihoensis var. mushaensis*, *Machilus zuihoensis var. zuihoensis*. Therefore, we choose these two types of galls on three plant species, which equal to six sample category as our material. [Fig. 4]

2.2.2 Plant Gall tissues processing

The freshly collected galls and plant stems including leaves are preserved in zipped bags and quickly sent to 4℃ refrigerator in laboratory. In no more than ten days after collection, we would cut these plant galls by dissection knife and pick out the larvae by needles. We also slice away some plant tissues which near the larvae, in order to reduce the chance of contamination. The processed plant gall tissues can be used in following experiment steps right away or preserved in -80℃ refrigerator for future use.

2.2.3 Plant Leaves processing

The healthy and qualified plant leaves including stems are preserved in zipped bags in

-80℃ refrigerator if not being used for 2-D electrophoresis right away. Preserve the stems alone with plant leaves is to maintain the freshness of leaves.

2.2.4 Plant tissue powder preparation

We adapted the method from Wang, 2003, Electrophoresis (Wei Wang, 2003). First we ground our sample tissue with liquid N_2 in stainless steel mortar and pestle. Then put 0.2g dry tissue powder into 2.0ml microtubes, added 1-2ml cold acetone vortexing thoroughly for 30s. The mixture was centrifuged at 10000x g for 3 min at 4℃. Pour out acetone and repeat the above-mentioned steps for 2-3 times. The pellet was moved into mortar and dried at room temperature. The dried tissue pellet was ground into finer powder by adding quartz sand and then transferred into new microtubes. The fine tissue powder was sequentially rinsed with 10% cold TCA/acetone 3-4 times or until the supernatant is colorless. The powder was following rinsed with 10% TCA/H_2O twice and cold 80% acetone twice. The pallet was vortexed and centrifuged as above-mentioned, and dried at room temperature. The dried powder can be use at following protein extraction and be stored at -80℃ refrigerator for future use.

2.2.5 Protein extraction & assay (phenol extraction) :

The dry tissue powder was resuspended in new 2.0ml microtubes with 0.8ml phenol buffer (Tris-buffered, pH 8.0, Sigma) and 0.8ml dense SDS buffer (30% sucrose, 2% SDS, 0.1M Tris-HCl, pH 8.0, 5% 2-mecaptoethanol), it was vortexed thoroughly for 30s then centrifuged at 10000x g for 3 min. The separated upper layer phenol was removed by pipette into fresh new microtubes, and be sure not to disturb the white interface SDS complex if there appeared any. At least 5-folds volume of cold 0.1M ammonium acetate/methanol was added into the phenol phase and stored in -20℃ refrigerator for 30 min. The precipitated proteins were centrifuged at 10000x g for 5 min to recover, and were poured out the upper layer cold ammonium/methanol. The pellet was sequentially washed twice each with cold ammonium/methanol and 80% acetone acetate. The protein precipitate was dried at room temperature and dissolve in 2-DE rehydration buffer. (8M urea, 2%CHAPS, 0.5% IPG buffer, 0.002% bromophenol blue stock solution). The concentration of protein extracts were estimated by RC DC protein assay kit (Bio Rad), following it's manual under 750nm.

2.2.6 2D-electrophoresis

2-DE was performed by a commercially available Ettan IPGphor IEF system and Hoefer SE600 Ruby (gel size 13cm x 15cm) from Amersham. The protein extracts were separated using gel strips and formed and immobilized nonlinear pH gradient from 3-10 (Immobiline Drystrip, pH3-10NL, 13cm, Amersham). Analytical IPG strips were rehydrated for 12h at 20℃ 30V with 250μL of the rehydration buffer including 100μg of protein extracts. IEF was performed at 20℃ in the Ettan IPGphor system (Amersham) for 1h at 500 V, 1h at 1000 V, 1h at 4000 V, and 2h at 8000V. Prior to the second dimension, the strips were equilibrated for 2 x 15 min in equilibration solution containing 6M urea, 75 mM Tris-HCl (pH 8.8), 29.3% v/v glycerol, 2% SDS, 0.002% bromophenol blue. DTT (1% w/v) was added to the first equilibration solution and 2.5 w/v iodoacetamide was added to the second one. For the second dimension, the strips were transferred onto SDS polyacrylamide gels (12.5%) with a run of 50mA *per* gel for 4-5h at 4℃. The 2DE gels were made in triplicate and sample proteins were from two independent extractions.

2.2.7 Protein staining and analysis of 2-DE gels

After electrophoresis, proteins were visualized by a modified silver-staining kit (Yan, J.S., 2000). Digital images of the gels were obtained by using an ImageScanner and were analyzed using ImageMaster 2D v3.1 elite software (Amersham). The spots were detected and the background was subtracted (mode: average on boundary), and the 2-DE gels were aligned and matched. A quantitative determination of the spot volumes was performed (mode: total spot volume normalization). Specific spots were described during different treatments when their volumes significantly differed (at least ten-fold in relative abundance). The interesting proteins were identified by ESI-Q-TOF-MS analysis.

2.2.8 Protein identification by MS

For MS analysis, protein spots were excised from the gel and digested with trypsin according to published procedures (Shevchenko, 1996). 34 labeled protein spots were sent to professor Chao-Hsiung Lin's laboratory in NYMU for Mass Spectrometric analysis. Proteins were identified by searching the protein databases NCBInr using MASCOT (http://www.matrixscience.com). To denote a protein as unambiguously identified, the Mowse scoring algorithms were sued. Only proteins whose score exceeded the significance threshold are discussed.

3. Result

3.1 Insect gene molecular evolution

In past studies, scientists regarded cecidomyiidae midges as highly host specific species (Harris, 1994). Cecidomyiidae midges are very fragile small insects usually only 2-3 mm. in length and many are less than 1 mm long. Unlike some gall-inducing insects of other plant species, most of Cecidomyiidae midges are unknown species and it's hard to classify them by their appearance. In order to solve this problem, we adopt the molecular classification method and use both COI and 12S mitochondria gene.

3.1.1 Gall Midge COI gene sequence alignment

In PileUp dendrogram, Distance along the vertical axis is proportional to the difference between sequences; distance along the horizontal axis has no significance at all. In fig 5, COI sequence alignment, we can see several major clusters. Since it's PileUp function in SeqWeb, the final output dendrogram is unrooted, and branch length has no meaning. There are 35 sample sequences and 3 out-group sequences. We choose these 3 out-group sequences from NCBI, All insects, which make same type of galls were grouped together. The first is constituted by same out-group species as 12s, *Asphondylia sphaera*, *Asphondylia gennadii*, and *Asphondylis itoi*. Second cluster is 3 blister morphospecies, which includes mj-blister56, mo-blister76, and mt-blister36. The third cluster includes 3 bulb morphospecies, they are mjk-bulb45, mj-bulb55, and m-bulb25. The forth cluster concludes 7 bullet morphospecies, which formed 3 small groups, each are mt-bullet39, mj-bullet59, mz-bulet10, mm-bullet29, mo-bullet79, mjk-bullet49, and mp-bullet69. The fifth cluster is made of 5 mice morphospecies, they are mj-mice51, mjk-mice41, mt-mice31, mz-mice11, and mm-mice21. The sixth cluster is mixed with 4 bell, 5 club, and 1 bird morphospecies. They are mt-bell32, mm-bell22, mjk-bell42, mt-club34, mo-club74, mp-club64, mk-bird83, mz-bell12, mj-club54, mjk-club44. Although in total they form a big cluster, but each different gall-making midges also grouped together to form smaller clusters. The seventh cluster contains 5 spindle morphospecies, which are mp-spindle68, mt-spindle38, mm-spindle28, mz-spindle18, and mj-spindle58and. The eighth cluster is 2 bud species, mz-bud17 and mt-bud37.

3.1.2 Gall midge 12s gene sequence alignment

In fig 6, 12s sequence alignment, we can see almost all the insects which make same type of galls were also grouped together. There are 28 sample sequences, 3 out-group sequences. All the sequences were clearly divided into 7 big clusters. The first cluster is 2 bud morphospecies grouped together, mt-bud37 and mz-bud37. The second cluster includes 4 spindle morphospecies, mz-spindle28 grouped with mm-spindle28; mj-spindle58, and mp-spindle68. The third cluster is 3 out-groups downloaded from NCBI, and these are also midges in the Cecidomyiidae family. The fourth cluster includes 3 bulb morphospecies, mm-bulb25 grouped with mj-bulb55, and mz-bulb15. The fifth cluster is 5 mice morphospecies, mjk-mice41 grouped with mt-mice31; mj-mice51 grouped with mz-mice11, and mm-mice21. The only exception is group six, which contains both 3 club morphospecies and 4 bell morphospecies while other cluster only contains same midges which made same types of galls. Sequences included in the sixth cluster are mj-club54, which grouped with bell12; mt-bell32 grouped with mjk-bell42 and mm-bell22; mo-clu74 grouped with mt-club34. The last cluster includes 7 bullet morphospecies, mt-bullet39 grouped with mj-bullet59; mz-bullet19 grouped with mz-bullet29; mp-bullet 69 grouped with mjk-bullet49 and mo-bullet79.

3.1.3 Gall midge COI gene evolutionary tree

The “Evolution” function in Seqweb investigates the evolutionary relationships within a group of sequences. It aligns a group of sequences, create a table of pairwise distances based on the aligned sequences, and create a tree graph representing the sequence relationships. In fig 7, COI gene evolutionary tree, the bar labeled with 10.00 at the bottom is branch length unit, it is a measure unit of all branch length. It means “10 substitutions per 100 residues”. There are 7 major clades and two independent sequences, which are mt-bud37 and mz-bud17. These insects which make same types of galls are grouped together.

The first clade consists of 3 out-group species, *Asphondylia sphaera*, *Asphondylia gennadii*, and *Asphondylis itoi*. Their sequence divergences are 12.94%, 14.13% and 16.02%.

The second clade consists of all blister morphospecies, which are mj-blister56, mt-blister36, mo-blister76. Their sequence divergences are 22.50%, 19.51%, and 27.95%. The third clade includes all spindle morphospecies, and it can be divided into two small clades. All sequence divergences in this clade range from 0.25% to 11.86%. The first small clade consist of mp-spindle68, mt-spindle38, and their sequence divergence is 0.25%. The second small clade consists of mm-spindle68, mz-spindle28, and mj-spindle58. Their sequence divergences are 0.51% and 2.05%.

The fourth clade consists of all bulb morphospecies, which are mjk-bulb45, mj-bulb55, mm-bulb25. The sequence divergences are 2.83%, 9.43%, 9.73%. The fifth clade consists of all bullet morphospecies, and it can be divided into three small clades. Sequence divergences among all sequences range from 0.51% to 6.65%. The first small clade consist of mz-bullet19, mm-bullet29, and their sequence divergence is 0.76%. The second small clade consists of mo-bullet79, mjk-bullet49, mp-bullet69, and their sequence divergences are 0.76%, 0.76%, and 0.51%. The third small clade consists of mt-bullet39, mj-bullet59, and their sequence divergence is 6.60%.

The sixth clade consists of all sequences of mice morphospecies, which are mj-mice51, mz-mice11, mjk-mice41, mt-mice31, and mm-mice21. Their sequence divergences are 0.25%, 2.58% and 2.84%. The seventh clade consists of three different gall-making midges, which are mt-bell32, mm-bell22, mjk-bell42, mz-bell12, mt-club34, mo-club74, mk-bird83, mp-club64, mj-club54, mjk-club44. The first three sequences are totally the same, and all sequence divergences range from 0.51% to 2.84%.

3.1.4 Gall midge 12s gene evolutionary tree

Same with 3.1.3 COI evolutionary tree, we used the "Evolution" function in SeqWeb to get our result. The bar labeled with 10.00 at the bottom is branch length unit, it is a measure unit of all branch length. It means "10 substitutions per 100 residues". In fig 8, 12s gene evolutionary tree, there are 28 sample sequences and 3 out-group sequences, witch constitute seven major clades.

The first clade is made of mt-bud37, mz-bud 17, and their sequence divergence is 0.61%. The second clade has 4 spindle sequences, mz-spindle18, mm-spindle28, mj-spindle58, mp-spindle68, the sequence divergence ranges form 0.31% to 8.12%. It can be divided into two small clades. The first clade consists of mz-spindl18, mm-spindle28, and the sequence divergence is 0.31%. The second clade consists of mj-spindle58, mp-spindle68, and the sequence divergence is 5.76%.

The third clade consisted of 3 out-group sequences, *Asphondylia sphaera*, *Asphondylia gennadii*, and *Asphondylis itoi*. Their sequence divergences are 14.88%, 15.89%, and 18.33%. The fourth group consists of three bulb morphospecies, they are mm-bulb25, mj-bulb55, and mz-bulb15. Their sequence divergences are 5.83%, 12.19%, and 16.68%. The fifth clade is bullet morphospecies, which includes mt-bullet39, mj-bullet59, mp-bullet69, mjk-bullet49, mo-bulle79, mz-bullet19, and mm-bullet29. They can be divided into three small clades, and all the sequence divergence ranges from 1.92% to 13.63%. The first small clade consists of mt-bullet49, mj-bullet59, and the sequence divergence is 2.61%. The second small clade consists of mp-bullet69, mjk-bullet49, mo-bllet79, and their sequence divergences are 0.95%, 1.92%, and 2.25%.

The sixth clade consists of 5 mice morphospecies, mjk-mice41, mt-mice31, mj-mice51, mz-mice11, and mm-mice21. Their sequence divergence ranges from 0.95% to 13.63%. It can also be divided into three small clades. The first small clade consists of mjk-mice41, mt-mice31, and the sequence divergence is 0.64%. The second small clade consists of mj-mice51, mz-mice11, and sequence divergence is 3.96%. The last clade is mm-mice21, the average sequence divergence between it among others is about 10%.

The last clade consists of bell- and club morphospecies, they are mt-bell32, mm-bell22, mjk-bell42, mo-club74, mt-club34, mj-club54, and mz-bell12. This clade can be divided further into two small clades, one consists of mt-bell32, mm-bell22, mjk-bell42, and sequence divergences range from 0.63% to 1.59%. The other group consists of mo-club74, mt-club34, mj-club54, and mz-bell12, and sequence divergences range from 2.89% to 4.87%.

3.1.5 Gall midge evolutionary relationships

Besides midge taxonomy, we also want to know the evolutionary relationships between each midge tribe, genus and species. Therefore, we use UPGMA method in evolutionary analysis and get an ultrametric tree. There are differences between NJ-tree and UPGMA-tree. First, ultrametric trees are rooted trees while NJ-trees are unrooted. Second, ultrametric trees are rooted trees in which all the end nodes are equidistant from the root of the tree. The branch length in NJ-tree indicates the genetic change and the relationships between taxa, but the branch length of an ultrametric tree is proportional to the divergent time. Therefore, we use UPGMA-tree to analyse the phylogeny and evolutionary relationship between gall midges.

In figure 9, COI gene evolutionary tree-UPGMA, we added another two Drosophila species as out-group besides the original 3 out-groups we used, in order to identify the root of all gall midges. Generally speaking, midges in the same clade make same type of galls. The root of gall midges is in the middle of blister morphospecies and other gall-making midges. In the compounded group of gall-making midges, the node divided into mt-bud37 and other gall-making midges, which forms one big clade. Then the big clade divided into two second big clades. One contains spindle morphospecies, mz-bud17, and out-group insects; the other clade contains bulb, bullet, mice, bell, club, and bird morphospecies. Then the bulb morphospecies divided from other gall-making midges. In the remaining clade, mt-bullet39 and mj-bullet59 divided from other gall-making midges. In the remaining clade, there are three major groups: bullet morphospecies, mice morphospecies and a last group, which contains bell, club, and bird morphospecies.

In figure 10, 12S gene evolutionary tree-UPGMA, we also added two Drosophila species as additional out-groups in order to determine the root of gall midges. The outcome is as same as COI gene evolutionary tree-UPGMA, midges in the same clade make same type of galls. The root, which means the ancestral species, diversified into two clades. The clade at right contains bud and spindle-making midges; the clade at left contains other gall making midges and the original out-groups. In the left clade, out-group species diversified first, then diversified bulb morphospecies. In the remaining clade, which contains mice, bell, club, and bullet morphospecies, divided into two little clades. The little clade at left contains all bullet morphospecies. The little clade at right divided into another two little clades, one is mice morphospecies; the other contains bell and club gall making midges.

3.1.6 Gall midge larvae morphology anatomy and gene analysis

In Dr. Tung's research in TFRI, the anatomical structures of all midge larvae can be classified into 4 types according to their spatula and anus. (Table 3, Table 4) In type I, there are two salient parts on larva's spatula and no terminal papillae on anus. In type II, the button part of larva's spatula is healed but the top of spatula is crotched. The shape of anus looks like a peach. In type III, the spatula shaped like chisel, and there are terminal papillae on anus. In type IV, the spatula is shorter than other type's spatula, but the middle part of it is bigger. The anus forms two segments but there is only one segment has papillae. Each larva in each type of galls can be classified into one type.

Among all four types of larvae, the type one larvae are the largest group. It contains all bulb-, bullet-, mice-, bell-, club-, and bird-gall-making-midges. Type two larvae only contains mt-bud37 midge. Type three larvae include mz-bud17, and all blister morphospecies. Type four larvae contain all spindle morphospecies.

We combine this morphological anatomy result with gene sequence analysis, (fig 5-8) and we also put sketches of gall types on this diagram.

From these diagrams, we can clearly see that midges with same anatomical structures were grouped together. In previous experiments, we know that midges which made same types of galls are closer species, or even same species. Therefore, the anatomical structures are the same among midges which made same types of galls.

3.2 Plant Proteomics

These plant proteomic experiments ware done by Hung-Pin Chen, my laboratory colleague. I collected and arranged his experimental data, and discuss these data together with my experimental data.

In order to analyse the impacts on which gall-inducing insects cause to plants, we used 2-dimensional electrophoresis technique to study the protein changes and differences between gall tissues, galled leaves, and un-galled leaves. First, we compared galled and ungalled leaves to determine whether gall-inducing insect caused damage to health leaves or not. Second, we compared the protein pattern differences between three *Machilus* leaves for excluding the original differences existed between different *Machilus* leaves at further comparisons. Third, we compared the protein pattern difference between two types of galls on single *Machilus* leaves to understand whether theses two types of galls caused different impacts or not. Then, we compared same type of galls but grow on different *Machilus*. At last, we compared two types of gall-tissues and *Machilus* leaf tissues on each *Machilus* in order to figure out the common protein differences between gall tissues and leaf tissues. After all the analyses of protein patterns, we combined the results of gall-insect taxonomy and discussed together.

3.2.1 Comparison of galled and ungalled leaves

In figure 12, (A) is 2D image of protein pattern of healthy leaf tissues and (B) is protein pattern of galled leaf tissues. Both samples are from *Machilus zuihoensis var. mushaensis* (MM). Healthy leaf tissue means that there are no galls or other damages on leaf surface. Galled leaf means that there are galls on leaf surface, but we took the ungalled part of leaf tissue.

3.2.2 Protein pattern differences between three *Machilus* leaves

After comparing protein differences between galled and ungalled leaves, we compared the protein pattern differences among three *Machilus* leaves. Botanists regard these three *Machilus* as different species, and we want to know whether there are protein differences between them. Then we can exclude these difference proteins among gall tissues on different *Machilus*, because these proteins are original differences between leaves.

In figure 13, (A) is the 2D image of *Machilus zuihoensis var. zuihoensis*. (MZ) leaf tissues (B) is 2D image of *Machilus zuihoensis var. mushaensis*.(MM) leaf tissues (C) is leaf tissues of *Machilus thunbergii*.(MT) Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other. We classified these three *Machilus* leaves into three groups, (A) and (B), (B) and (C), (A) and (C), then we compared leaf protein patterns with each other in each group.

As figure 13 shows, in group (A) (B), the different proteins are protein No. 1. in MT-leaf tissue (A), and protein No. 2, 3, 4, 5 in MM-leaf tissues (B). There are total 5 different proteins in this group.

In group (A) (C), the different proteins are protein No. 6-11 in MT-leaf tissues (A) and protein No. 12-25 in MZ-leaf tissues (C). There are total 20 different proteins in this group.

In group (B) (C), the different proteins are protein No.2, 4, 5, 6-11 in MM-leaf tissues (B) and protein No. 1, 12-25 in MZ-leaf tissues (C). There are total 24 different proteins in this group.

In figure 14, we made a three-circle graph of original protein differences among three *Machilus* leaves. One circle represents one *Machilus* species, and in each circle are their own proteins. We can clearly see that the protein differences between MZ and MM leaves are smaller than the differences between MM-MT, and MZ-MT. The protein patterns of MZ and MM leaves are alike, there are 6 proteins in both MZ and MM leaves which don't appear in MT leaves (protein 6-11). There are 14 proteins, which only appear in MT leaves (protein 12-25). Protein 2, 4, and 5 only appear in MM leaves. There are no distinctive proteins which only appear in MZ leaves.

3.2.3 Protein pattern differences between two types of gall on single *Machilus* leaves

In this experiment, we analyzed the protein pattern differences between two types of gall on single *Machilus* leaves. In figure 15, each graph are 2D images of our samples. Samples were classified into three groups according to three *Machilus* species: (D) and (G), (E) and (H), (F) and (I). There are two types of galls in each group, bell gall and mice gall. (D) is bell-gall tissues of *Machilus zuihoensis var. zuihoensis*. (MZ-bell). (E) is bell-gall tissues of *Machilus zuihoensis var. mushaensis.* (MM-bell). (F) is bell-gall tissues of *Machilus thunbergii.* (MT-bell) (G) is mice tissues of *Machilus zuihoensis var. zuihoensis*. (MZ-mice). (H) is mice-gall tissues of *Machilus zuihoensis var. mushaensis.* (MM-mice). (I) is mice-gall tissues of *Machilus thunbergii.* (MT-mice).The protein spots labeled are those who differentially expressed by at least ten-fold in comparison with each other.

In group (D) (G), bell and mice galls on MZ, the different proteins are protein No. 34, 104, 105 in MZ-bell (D) and protein No. 29, 70, 71 in MZ-mice (G). There are total 6 different proteins in this group.

In group (E) (H), bell and mice galls on MM, the different proteins are protein No. 5, 37, 70 in MM-bell (E) and protein No. 34, 46 in MM-mice (H). There are total 5 different proteins in this group.

In group (F) (I), bell and mice galls on MZ, the different proteins are protein No. 65, 95, 102 in MT-mice (I), and there are no special protein in MT-bell (F). There are total 3 different proteins in this group.

As the result shows, there are little protein pattern differences between bell and mice galls, which grow on same *Machilus* species.

3.2.4 Protein pattern differences between bell galls on three *Machilus* leaves

Next, we compared the protein pattern difference between bell galls on three *Machilus* leaves in order to see whether *Machilus* species affects gall tissues proteins. The bell-galls of three *Machilus* were classified into three groups, (D) and (E), (D) and (F), (E) and (F). In Fig. 16 and table 9, the bell-gall protein patterns were compared with each other. (D) is bell gall tissue of *Machilus zuihoensis var. zuihoensis*. (MZ-bell) (E) is bell gall tissues of *Machilus zuihoensis var. mushaensis.*(MM-bell)**.** (F) is bell gall tissues of *Machilus thunbergii.* (MT-bell). Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

In group (D) (E), bell galls on MZ and MM, the different proteins are protein No. 34, 104, 105 in MZ-bell (D), and protein No, 29, 70, 71 in MM-bell (E). There are total 6 different proteins between bell gall tissues on MZ and MM.

In group (D) (F), bell galls on MZ and MT, the different proteins are protein No. 1, 11, 31, 32, 34, 37, 42, 84, 104, 105 in MZ-bell (D) and protein No. 22, 70, 92, 94, 103, 106 in MT-bell. There are total 16 different proteins between bell gall tissues on MZ and MT.

In group (E) (F), bell galls on MM and MT, the different proteins are protein No. 1, 11, 32, 37, 42, 71, 84, 87 in MM-bell (E) and protein No. 2, 92, 94, 103, 106 in MT-bell (F). There are total 13 different proteins between bell gall tissues on MM and MT.

3.2.5 Protein pattern differences between mice galls on three *Machilus* leaves

After comparing bell galls on three *Machilus* leaves, now we compared the protein pattern differences between mice galls on three *Machilus* leaves, in order to understand whether the effects of these two galls caused to plants are the same or not. In Fig. 17 and table 10, the mice galls of three *Machilus* were classified into three groups, (G) and (H), (G) and (I), (H) and (I). The 2D images of mice gall protein patterns were compared with each other. (G) is mice galls of *Machilus zuihoensis var. zuihoensis*. (MZ-mice). (H) is mice galls of *Machilus zuihoensis var. mushaensis.* (MM-mice). (I) *is* mice galls of *Machilus thunbergii.*

(MT-mice). Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

In group (G) (H), the different proteins are protein No. 1 in MZ-mice (G) and protein No. 4, 46, 59 in MM-mice (H). There are total 4 different proteins between mice gall tissues on MZ and MM.

In group (G) (I), the different proteins are protein No. 1, 11, 43, 45, 71, 84, 87 in MZ-mice gall tissues (G) and protein No. 12, 22, 62, 65, 89, 94, 102, 103, 106 in MT-mice gall tissues. There are total 16 different proteins between mice gall tissues on MZ and MT.

In group (H) (I), the different proteins are protein No. 4, 11, 34, 45, 59, 71, 84, 87 in MM-mice (H) and protein No. 22, 64, 65, 70, 94, 102, 103, 106 in MT-mice (I). There are total 16 different proteins between mice gall tissues on MM and MT.

3.2.6 Common protein pattern differences between gall tissues and leaf tissues

3.2.6.1 Protein pattern differences between bell-gall-tissues, mice-gall-tissues, and leaf tissues of *Machilus zuihoensis var. zuihoensis*. (MZ)

In Fig 18, we compared the protein pattern differences between each bell-gall-tissues and mice-gall-tissues with healthy leaf tissues of MZ. Photographs (D), (G), (A) are 2D images. (A) is leaf tissues of *Machilus* zuihoensis var. zuihoensis. (MZ-leaf). (D) is bell gall tissues of *Machilus* zuihoensis var. zuihoensis. (MZ-bell). (G) is mice gall tissues of *Machilus* zuihoensis var. zuihoensis. (MZ-mice). Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

In group (A) (D), the different proteins are protein No. 1, 11, 26-42 in MZ-bell-gall-tissues (D) and protein No. 6, 10, 43-69 in healthy MZ leaf tissues (A). There are total 48 different proteins between bell-gall-tissues and healthy leaf tissues.

In group (A) (G), the different proteins are protein No. 1, 11, 26-29, 35, 36, 39, 70-72 in MZ-mice-gall-tissues (G) and protein No. 6, 10, 43-69, 73-75 in healthy MZ leaf tissues (A). There are total 44 different proteins between mice-gall-tissues and healthy leaf tissues.

3.2.6.2 Protein pattern differences between bell-gall-tissues, mice-gall-tissues, and leaf tissues of *Machilus zuihoensis var. mushaensis.* (MM)

In Fig 19, we compared the protein pattern differences between each bell-gall-tissues and mice-gall-tissues with healthy leaf tissues of MM. Photographs (E), (H), and (B) are 2D images. (B) is leaf tissues of *Machilus* zuihoensis var. mushaensis. (MM-leaf). (E) is bell galls of *Machilus* zuihoensis var. mushaensis. (MM-bell). (H) is mice galls of *Machilus* zuihoensis var. mushaensis. (MM-mice). Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

In group (B) (E), the different proteins are protein No. 1, 11, 26-29, 32, 36-38, 70-72, 76-87 in MM-bell-gall-tissues (E) and protein No. 3-6, 10, 43-46, 52-56, 58-63, 65, 66, 68, 69 in healthy MM leaf tissues (B). There are total 49 different proteins between bell-gall-tissues and healthy leaf tissues.

In group (B) (H), the different proteins are protein No. 11, 13, 14, 26-29, 70, 71, 79, 80, 83, 84, 86, 88, 89 in MM-mice-gall-tissues (H) and protein No. 3, 5, 6, 10, 43-46, 48, 53-56, 58-62, 65, 66, 68-91 in healthy MM leaf tissues (B). There are total 40 different proteins between mice-gall-tissues and healthy leaf tissues.

3.2.6.3 Protein pattern differences between bell-gall-tissues, mice-gall-tissues, and leaf tissues of *Machilus thunbergii.* (MT)

In Fig 20, we compared the protein pattern differences between each bell-gall-tissues and mice-gall-tissues with healthy leaf tissues of MT. Photographs (F), (I), and (C) are 2D images. (C) is leaf tissues of *Machilus* thunbergii. (MT-leaf). (F) is bell gall tissues of *Machilus* thunbergii. (MT-bell). (I) is mice gall tissues of *Machilus* thunbergii. (MT-mice). Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

In group (C) (F), the different proteins are protein No. 22, 26, 27, 33, 36, 70, 76, 92-94, 103 in MT-bell-gall-tissues (F) and protein No. 1, 3, 15, 16, 19. 43-46, 50, 52-67, 69, 74, 95-98 in healthy MT leaf tissues (F). There are total 42 different proteins between bell-gall-tissues and healthy leaf tissues.

In group (C) (I), the different proteins are protein No. 26, 27, 29, 36, 70, 72, 73, 76, 92-94, 99-103 in MT-mice-gall-tissues (I) and protein No. 1, 3, 15, 16, 19, 43-46, 50, 52-62, 64-67, 69, 74, 95-98 in healthy MY leaf tissues (C). There are total 47 different proteins between mice-gall-tissues and healthy leaf tissues.

Total protein differences between each sample is displayed in Table 10.

4. Discussion

Cecidology is the study of plant galls. It crosses multiple areas such as ecology, animal taxonomy, botanical taxonomy, botanic pathology, parasitology, and it's a special research subject. In Taiwan, *Machilus* (Lauraceae) plants have the widest variety of gall shapes among all plant species. Cecidomyiidae (Dipteria) is the major gall inducing insects on *Machilus*.

There are many unsolved mysteries in Cecidology, including insect taxonomy, gall growth mechanisms, relationships between insects and plants…etc. In order to have further solutions to these questions, we approached from both insect genomics and plant proteomics.

4.1 Insect gene molecular evolution

In past studies, scientists regarded cecidomyiidae midges as highly host specific species (Harris, 1994). Because of host specificity characteristics of insects, biologists regard the phylogeny relationship between gall-inducing midges on *Machilus* is closer among species which live on same host, but there are no evidences to prove it. Moreover, all the midges looked the same; we can't identify them by morphology.

Therefore, we adopt the method called "DNA barcode." The basic concept of this method is that every species has its' unique DNA sequences, which can be regarded as biological barcodes which we can use to identify them. Among all insect genes, there is one generally acknowledged gene used as the "barcode gene" by most entomologists and molecular scientists, the COI gene. There was an order gene which served as the "barcode gene" before, but was then founded to have several defects and be abandoned. In this research, we used these two genes, and will discuss the differences between these two results.

This research reveals that the phylogeny relationship is closer between species which make same type of galls. It might because gall midges secrete chemicals which induce galls on host plants, and the closer species secrete same chemicals and induce same galls.

4.1.1 Gall midge COI gene sequence alignment

In figure 5, midge COI gene sequence alignment, we can see that all midges which make the same type of galls are grouped together. It means that gall midges which make same type of galls, although live on different *Machilus* species, have more similar COI genes to each other than midges which make different types of galls but live on the same *Machilus* species. Midges which have more similar COI genes can be regarded as more phylogenetically related. The three out-group midges we chose are clearly divided from sample midges.

This situation can be apply to midges which make bud, bullet, bulb, blister, mice, and spindle galls, but there's a little exception among midges which make bell, club, and bird galls. In fig 5, the COI gene sequence of midges which make bell, club, and bird galls are grouped together, but there are small groups in this big group, most COI genes of bell morphospecies are still more similar to each other, and so are COI genes of club morphospecies. Bird morphospecies is grouped between bell- and club morphospecies, which means bird morphospecies might has closer phylogenetic relationship with these two midges.

4.1.2 Gall midge 12S gene sequence alignment

Although scientists don't use 12S gene for DNA phylogenetic analysis nowadays, we can still check the differences between the results of 12S gene and COI gene. In fig 6, midge 12S gene sequence alignment, the result is the same as that of COI gene alignment. All midges which make the same type of galls are grouped together. It also supports our result in 4.1.1: Gall midges which make same type of galls, although live on different *Machilus* species, have more similar COI genes to each other than midges which make different types of galls but live on the same *Machilus* species. Midges which have more similar 12S genes can be regarded as more phylogenetically related. The three out-group midges we chose are clearly divided from sample midges.

As the result of COI gene, the result is also true to midges which make bud, bullet, bulb, blister, mice, and spindle galls, and there's also a little exception among midges which make club galls (we failed to collect bud and bird galls). In fig 6, the 12S gene sequence of midges, midge mj-club54 is grouped together with bell morphospecies but separated from other club morphospecies. But if we looked a little more widely, both club and bell morphospecies formed a big group. This situation also happened in COI gene alignment.

4.1.3 Gall midge COI gene evolutionary tree

In previous research, the average COI gene divergence between congeneric moth is 6.05%, and 7.93% between congeneric bird. The COI gene divergence between conspecies moth is 0.25% and 0.27% between conspecies bird (Hebert, 2003.) In Mehrdad H. et al's research (2006), the COI gene divergence within family Lepidopteran is 11.26%, COI gene divergence of congeneric Lepidopteran is 6.8%, and COI gene divergence of conspecies Lepidopteran is 0.25%. Also in Hebert's research (Hebert, 2003), he suppose that all living species in animal kingdom have similar gene divergence in one specific gene; the divergence situation is also called "gene barrier". Therefore, in my research, I set the gene barrier of gall-making midges in consideration of all the data above and my gene analysis outcome. These barriers are: 0.25%-6% for conspecies Dipteria midges; 6%-11% for congeneric midges; 11%-17% for midges in same tribe; 17% and above for midges in different tribe but in same subfamily. The reason why I set such detailed gene divergence for tribe and subfamily is that in the taxonomic tree in NCBI, tribe and subfamily are listed between common known classification, genus and family. The sequential relationships from high to low are family, subfamily, tribe, genus, and species.

In my analysis, according to COI gene divergence analysis, 35 sample Cecidomyiidae midges are in the same subfamily and can be divided into 7 tribes, 11 genus and 15 species (table. 5.)

The first tribe T1 is MZ-bud17 midge, which forms its own genus G1 and species S1. The second tribe T2 is MT-bud37 midge, which also contains a genus G2 and species S2. The third tribe T3 is mt-blister36 midge, and it also forms its own genus G3 and species S3. The fourth tribe T4 is mj-blister56 midges, and it forms a genus G4 and species S4 either. The fifth tribe T5 is mo-blister76 midges, like previous four tribes, it also forms its own genus G5 and species S5.

The sixth tribe T6 is spindle tribe, which contains all spindle morphospecies. There is one genus and two species in spindle tribe. Genus G6 contains two species S6 and S7. Although the first species in spindle tribe S6 is one species, it contains mt-spindle38 midges and mp-spindle68 midges. It means that this midge species made spindle galls on both mt and MP trees. The second species in G6 is S7, which contains three spindle morphospecies: mm-spindle18, mz-spindle28, mj-spindle58 midges.

The seventh tribe T7 comprises 5 genus, G7-G11, and 8 species, S8-S15. Unlike those tribes mentioned before, this compounded tribe consists of several kinds of gall-making midges which make different types of galls. This tribe includes bullet, bulb, bell, mice, club, and bird types of galls. Each type of gall-making midges forms a genus, except for bell, club, and bird morphospecies.

Genus G7, the bulb morphospecies genus, contains two species, S8 and S9. Species S8 consists of mjk-bulb45 and mj-bulb55 midge, and it implies that this midge species made bulb galls on both MJK and MJ trees. Species S9 has only one sample midges, mm-bulb25 midges.

Genus G8 and G9 can be called "the bullet-gall making midge genus", these two genus are formed by all bullet morphospecies. Genus G8 contains two species, S10 and S11. Although these are all spindle morphospecies, they can be divided into two different species. S10 are formed by two sample midges, mz-bullet19, and mm-bullet29 midges. It represents that this kind of midge made bullet gall on mz and Mm trees. S11 contains three sample midges: mjk-bullet49, mp-bullet69, mo-bullet79 midges. This result also demonstrates that this midge species made galls on three *Machilus* trees: mjk. MP, and mo. Genus G9 contains two species, S12, S13. Each species is formed by one kind of midge. S12 is mt-bullet39 midge and S13 is mj-bullet59 midge.

Genus G10, "the mice morphospecies" genus, has only one species, but it contains 5 different sample midges. S14 contains mz-mice11, mm-mice21, mjk-mice31, mj-mice41, mt-mice51 midges. It also represents that this midge species made galls on 5 different *Machilus* species.

The last genus in compounded T7 tribe is Genus 11. It is also a compounded genus. S15 is the only species in this genus, but there are several different sample midges in it. These sample midges are: mz-bell 12, mm-bell 22, mt-bell 32, mjk-bell 42, mt-club 34, mjk-club 44, mj-club 54, mp-club 64, mo-club 74, Mk-bird 83. This is an interesting result, because every other species made only one type of galls, but this midge species made several different kinds of galls.

Although NJ-tree is an unrooted tree, we can estimate the phylogenic relationships between midge species by gene differences. Gene would mutate during evolution, and as the mutations accumulate, gene difference would become larger and larger. If one species has larger gene differences with other species, it might be an older species.

In our analysis, the oldest midge species might be bud morphospecies, since it form two different tribes and has the largest gene differences. The second old species might be blister morphospecies. The common ancestral blister morphospecies differentiated in to three genera and three species. The third old midge species might be spindle morphospecies. These midge forms one genus and two species. The fourth differentiated midge species are bulb morphospecies. They formed one genus and two species. The fifth differentiated midge species are bullet morphospecies, they formed two genus and three species. The sixth differentiated midge species are mice morphospecies. They formed one genus and one species.

The last differentiated species are compounded species. The remained ancestral midge species differentiated into three different gall-making midges. These three gall-making midges are bell morphospecies, club morphospecies, and bird morphospecies. They are all in same species, and gene differences are the smallest.

4.1.4 Gall midge 12S gene evolutionary tree

Besides COI gene divergence analysis, I also analyzed the phylogeny relationship of midge by using ribosomal 12S gene. In past researches, scientists chose 12S gene as the major target gene for phylogeny analysis, but there are several defects of 12S gene. First, its broad use in broad taxonomic is constrained. Second, the insertions and deletions are common in 12S gene. The reasons why we still did this experiment is to see the difference between two evolutionary trees.

Since we ran out of some samples during our last experiment, there are only 28 sample species left. These 28 sample Cecidomyiidae midges can be classified into 6 tribes 12 genus and 16 species. (table. 6.)

The first tribe T1 contains all bud species, which forms its own genus G1-bud and species S1. S1 contains two samples, mt-bud37 and mt-bud17. The second tribe contains all spindle morphospecies, and contains 3 species, S2 to S4. Species S2 includes mz-spindle18 and mm-spindle28. Species S3 is mj-spindle58, and species S4 is mp-spindle 68.

The third tribe T3 contains all bulb-gall making midges. It contains two genera, G3-bulb-1 and G4-bulb-2. G3-bulb-1 includes one species which has two bulb morphospecies, mm-bulb25 and mj-bulb55. G4-bulb-2 has one species S6, mz-bulb15.

The fourth tribe T4 contains all bullet morphospecies. It contains tree genera, G5-bullet-1, G6-bullet-2 and G7-bullet-3. G5-bullet-1 contains one species S7. S7 consists of two gall midges, mt-buleet39 and mj-bullet59. G6-bullet-2 contains one species S8. S8 consists of three gall midges, mp-bullet69, mjk-bullet49, and mo-bullet79. G7-bullet-3 contains one species S9, and S9 consists of two gall midges, mz-bullet19, mm-bullet29.

The fifth tribe T5 is mice tribe. It contains three genera, G8-mice-1, G9-mice-2, and G10-mice-3. G8-mice-1 has one species, S10, which contains two gall midges, mjk-41 and mt-mice31. G9-mice-2 also has one species, S11, which contains two gall midges, mj-mice51, and mz-mice11. G10-mice-3 has one species, S12, which contains only one gall midge, mm-mice21.

The sixth tribe T6 is a compounded tribe, has 2 genera and 4 species. It consists of several different gall-making midges, which includes bell morphospecies and club morphospecies. G11-bell contains one species, S13, which includes three bell-gall-midges: mt-bell32, mm-bell22, and mjk-bell42. G12-club bulb contains three species: S14, S15, and S16. There are two club-gall-making species in S14, mo-club74 and mt-club34. S15 only has one club morphospecies, mj-club54. S16 also has only one gall-making midges, mz-bell12.

The phylogeny relationships of 12S gene analysis are a little bit different with the results of COI gene analysis. Part because that we ran out of some samples when analyzed 12S gene. (In 12S, there are no mj-bliseter56, mt-blister36, mo-blister76, mt-spindle38, mjk-bulb45, mk-bied83, mp-club64, mjk-club44) But midges which made same galled are also classified into the same clade, like the result of COI gene analysis. The only little differences are how species are classified.

As in COI gene analysis, Gene would mutate during evolution, and as the mutations accumulate, gene difference would become larger and larger. If one species has larger gene differences with other species, it might be an older species.

In this experiment, according to phylogenetic tree (fig. 8), the oldest midge species might be bud morphospecies, since it form two different tribes and has the largest gene differences. Since we ran out of blister morphospecies sample while doing 12S gene analysis, the second old species might be spindle morphospecies. These midge forms one genus and three species. The third differentiated midge species are bulb morphospecies. They formed two genus and two species. The fourth differentiated midge species are bullet morphospecies, they formed three genus and three species. The fifth differentiated midge species are mice morphospecies. The little difference is that in COI gene analysis, all mice morphospecies are the same species, but in 12S gene analysis, mjk-41 and mt-mice31 form species S10; mj-mice51, and mz-mice11 form S11; mm-mice21 form S12. They formed three genus and three species. The last differentiated species are compounded species. Since we also ran out of bird morphospecies samples in this experiment, in comparison with COI gene analysis, there are only two different gall-making midges in this species. These three gall-making midges are bell morphospecies, and club morphospecies. They are all in same species, and gene differences are the smallest.

4.1.5 Evolutionary relationships of cecidomyiidae midges

In the evolutionary relationship analysis, we constructed evolutionary trees by using the UPGMA method to analyse cecidomyiidae genes.

The UPGMA is the simplest method of tree construction. It was originally developed for constructing taxonomic phenograms, but it can also be used to construct phylogenetic trees if the rates of evolution are approximately constant among the different lineages. For this purpose the number of observed nucleotide or amino-acid substitutions can be used. UPGMA employs a sequential clustering algorithm, in which local topological relationships are identified in order of similarity, and the phylogenetic tree is built in a stepwise manner.

The basic concept of UPGMA method is to assume the rates of evolution are approximately constant among the different lineages, which also means a molecular clock. Therefore, the branch lengths of an ultrametric tree are proportional to the divergent time.

In figure 9, midge COI gene evolutionary tree-UPGMA, the two Drosophila out-group species are much older than the oldest ancestral midge species. The oldest ancestral midge species then differentiated into the ancestral blister morphospecies species and the ancestral species of other gall-making midges.

The second differentiated midge is mt-bud37 midge, but the other bud morphospecies differentiated a little later. At this stage, the ancestral midge diversified into two generally classified clades: the leaf morphospecies and stem-gall making midge, and stem morphospecies were differentiated earlier than leaf morphospecies.

The third differentiated midge is mz-bud17 in the stem morphospecies clade, and later came the spindle morphospecies. When the spindle morphospecies diversified in the stem morphospecies clade, the ancestral midge in the leaf morphospecies clade were also diversifying. The first differentiated midge species in this clade are bulb morphospecies, which differentiated almost at the same time with spindle morphospecies. Later in leaf morphospecies, 2 in 7 bullet morphospecies differentiated first: mt-bulle19 and mj-bullet29. The remained 5 bullet morphospecies differentiated later with other gall-making midges.

The remained clade diversified into three little clades: the bullet morphospecies clade, mice morphospecies clade, and the last clade which contains bell-, club- and bird morphospecies. The remained 5 bullet morphospecies differentiated first among these three clades. The second differentiated are all mice morphospecies. The last differentiated clade contains bell-, club-, and bird morphospecies. This clade can also be divided into three little clades. One contains all bell morphospecies; second contains mt-club34, mo-club74, and mk-bird83. The last little clade contains mp-club54, mj-club64, and mjk-club44. There three little clades differentiated at almost the same time.

In another experiment, fig. 10, 12s gene evolutionary tree-UPGMA, the result is only a little different in comparison with the result of COI gene. Since there are several defects while using 12S gene, sciences nowadays don’t use 12S gene for gene analysis. Therefore, the result of 12S gene evolutionary tree-UPGMA is regarded as a reference.

Because of ran out of blister morphospecies samples when doing 12S gene analysis, there are no blister morphospecies in this evolutionary tree. The oldest ancestral midge species differentiated into two clades. Generally speaking, these two clades are the stem morphospecies clade and leaf morphospecies clade; stem morphospecies are differentiated earlier than leaf morphospecies. In the stem morphospecies clade, the ancestral species of both spindle morphospecies and bud morphospecies differentiated very early, but both ancestral species diversified much later.

In the leaf morphospecies clade, the first differentiated species are out-group species, than differentiated bulb morphospecies. The other ancestral species differentiated into three little clades: mice morphospecies clade; bullet morphospecies clade; bell- and club morphospecies clade. Among these three little clades, bullet morphospecies are first differentiated; mice morphospecies differentiated second. The bell- and club morphospecies differentiated the latest.

The outcome of 12S gene evolutionary tree-UPGMA is almost the same with COI gene analysis. Generally speaking, stem morphospecies differentiated from ancestral species earlier than leaf morphospecies.

4.1.6 Gall midge larvae morphology anatomy and gene analysis

From figure 5 to 8, we can clearly see that midges which made same types of galls are classified together in same clade, and the anatomical structures of these larvae are also the same.

In previous evolutionary analysis, blister morphospecies might be the oldest midge species. The second old species is bud morphospecies. The next differentiated midge species are spindle morphospecies, then came bulb morphospecies. The next generated midge species are bullet morphospecies and then mice morphospecies. The last differentiated midge species are bell-, club-, and bird morphospecies.

The anatomical structure of larvae can be classified into four different types according to their traits in spatula and anus. In these four types of larvae, the type I larvae are the largest group. It contains all bulb-, bullet-, mice-, bell-, club-, and bird-gall-making-midges. Type II larvae only contains mt-bud37 midge. Type III larvae include mz-bud17, and all blister morphospecies. Type IV larvae contain all spindle morphospecies. In insect morphology phylogeny, type III larvae are regarded as the oldest form of larvae morphon. Type II is the second oldest form of larvae morphon. Type IV is the third old form of larvae morphon, and type I is the latest differentiated form of larvae morphon.

If we combine larvae morphology and gene phylogeny analysis, we can find out that there is coherence between these two. In gene analysis, the oldest midge species is blister morphospecies, and their type III larvae also has the oldest anatomical structure. The second oldest midge species is bud morphospecies, and their type III & II larvae are also the oldest & second oldest. The third oldest midge species are spindle morphospecies, and their type IV larvae also has the third oldest anatomical structure. Other midge species, the bullet-, bulb-, mice-, bell-, club-, and bird morphospecies are all later generated species, and their type I larvae are also regarded as the latest generated larvae types.

4.1.7 Summary of gall midge taxonomy relationships

According to plant taxonomy and phylogeny, generally speaking, galls which grow on stems are usually regarded earlier forms of galls, and galls which grow on leaves are regarded later forms of galls.

For instance, cecidologists believe that blister gall might be the oldest form of all galls, because it has no vivid shape, it is only an irregular space between top and down layers of a leaf. There’s no further tissue development or differentiation. Midge larva lives inside and several larvae might share one blister gall.

The second old form of galls might be spindle, because it forms inside stem and also has irregular shape. There are several long and narrow spaces inside one spindle gall, and in each space live several midge larvae. The third old form of galls might be bullet and/or bud galls. These two forms of galls are abnormal growth of leaf-like tissues on stems. There are two forms of bud galls, one is long bud and the other is round bud. There may be several midge larvae inside long bud gall, but there is only one larva inside round bud gall.

About leave galls, there's no definite timelines of what form came out first and what came late, but leave galls came out later than stem galls.

In our experiment results, midges which made stem-galls are earlier species, which is identical to the result of gall shape taxonomy. The oldest species of gall midge are those made bud galls. Since they are the oldest midge, they differentiated into two tribes. The second old species are midge which made blister galls, and they differentiated into three different tribes. The third old species are midges which made spindle galls, and they are in the same tribe but differentiated into two species. The latest midge tribe made several different galls, which include one stem gall and five leaf galls. The only stem gall is bullet gall, and the 5 leave galls are bulb, mice, bird, club, and bird.

These midges form 5 genus and 8 species. There are no vivid taxonomy timeline among these different species, but midges which made same type of galls are classified into same genus. The 5 genus are G7-bulb, G8 & G9 bullet, G10 mice. The latest midge species made three forms of galls: bell, club, and bird.

Since all midges look the same, it's hard to distinguish and classified them only by their figures. And also because some gall-inducing insects are highly host specific, some entomologists think Cecidomyiidae midge might be highly host specific. It means that they think midges which made galls, no matter what form, are same species.

In our experiment, we prove that midges which made same types of galls, no matter on what species of *Machilus*, are closer to each other in phylogeny. It also means that same or closer midge species secret similar chemicals to induce same galls on different *Machilus*. The taxonomy relationship has nothing to do with host specificity.

Not only gall taxonomy and midge gene phylogeny are coherent, larvae morphology phylogeny and gene phylogeny are also coherent. (see discussion 4.1.6), and these reveals that gene evidences, morphological evidences, and ecological evidences can support each other.

4.2 Plant Proteomics

4.2.1 Comparison of galled and ungalled leaves

As figure 12 shows, there are no protein differences between galled and ungalled leaves. It represents that galls don't affect proteins of surrounding leaf tissues. It also means the gall-inducing insects only affect certain areas on leaves (where they inject chemicals), but won't affect leaf parts other then galled areas. The growth of galls won't effect the protein patterns of host plants.

4.2.2 Protein pattern differences between three *Machilus* leaves

In figure 13, we made a three-circle graph of original protein differences among three *Machilus* leaves. One circle represents one *Machilus* species, and in each circle are their proteins. We can clearly see that the protein differences between MZ and MM leaves are smaller than the differences between MM-MT, and MZ-MT. The protein patterns of MZ and MM leaves are alike, there are 6 proteins in both MZ and MM leaves which don't appear in MT leaves (protein 6-11). There are 14 proteins, which only appear in MT leaves (protein 12-25). Protein 2, 4, and 5 only appear in MM leaves. There are no distinctive proteins which only appear in MZ leaves.

The result of protein patterns could support the result of botany and plant taxonomy. Botanists in Taiwan regard MZ and MM are closer species, some botanists regard them as same species, but others don't. But one thing that can be sure is that MZ and MT, or MM and MT are definitely different species. Our result, the protein pattern differences are smaller between MZ and MM in comparison with the differences between each and MT, can prove that although MZ and MM are not quite the same, but are not completely different species neither.

4.2.3 Protein pattern differences between two types of gall on single *Machilus* leaves

In this experiment, we compared the protein pattern between bell and mice galls on same *Machilus* leaves. In each group, there are only slight protein differences. There are only 6 different proteins in group MZ-bell and MZ-mice; 5 different proteins in group MM-bell and MM-mice, but there are only 3 different proteins between MT-bell and MT-mice. From this result, we can have two conclusions.

First, the relevance between protein and the shape of galls is small, but these proteins might relate to shape changes. The other evidence is that the shape of MT-bell is different with other bell galls. The shape of MT-bell is round and smooth. In Chapter 2 fig 4, we can clearly see that rather than having crest lines on the surface like other bell galls, the shape of MT-bell looks more like mice galls without pili. In our result, the protein differences are also smaller between MT-bell and MT-mice. Therefore, these proteins might relate to gall shapes.

The second conclusion is that, the shape of galls might depend on insect species by chemicals they secreted, not host plant species.

4.2.4 Protein pattern differences between bell galls on three *Machilus* leaves

Next we compared the protein pattern of bell gall tissues on three *Machilus* species. In figure 16 and table 8 there are 6 different proteins between MZ-bell and MM-bell, but there are 16 different proteins between MZ-bell and MT-bell; 13 different proteins between MM-bell and MT-bell. The reason why protein pattern differences are smaller between MZ-bell and MM-bell might because these two *Machilus* are closer species, and they reacted much more similar to chemicals which insect secrets. Therefore, MZ-bell and MM-bell has similar protein patterns.

4.2.5 Protein pattern differences between mice galls on three *Machilus* leaves

As same as previous experiment, we compared the protein pattern difference between mice galls on three *Machilus* species. In figure 17 and table 9, there are 4 different proteins between MZ-mice and MM-mice; but there are 16 different proteins between MZ-mice and MT-mice; 16 different proteins between MM-mice and MT-mice. The results are the same as previous experiment in 4.2.5. Because MZ and MM are closer species, they reacted more similar to chemicals which insect secreted.

4.2.6 Common protein pattern differences between gall tissues and leave tissues

In this section, we compared the protein pattern differences between two types of gall tissues with healthy leave tissues. By doing this experiment, we can determine whether there are consensus protein patterns or not.

Although the protein differences are large in each group, if we looked at all 2D images, we can find out that there are several proteins in common within all groups.

In figure 21, we divided these proteins into four groups according to their related positions on 2D gels. Group "a" are proteins which were increased or induced in gall tissues; while group "b", "c", and "d" are proteins which were decreased or vanished in gall tissues (or proteins which only appears in healthy leaf tissues).

4.2.6.1 Protein pattern differences between bell-gall-tissues, mice-gall-tissues, and leaf tissues of *Machilus zuihoensis var. zuihoensis*. (MZ)

In figure 18, MZ-bell gall tissues (D) were compared with MZ-leaf (A), there are 3 proteins in group a in MZ-bell, protein 26, 27, 28. And in MZ-leaf, there are 4 proteins in group b, protein 43, 44, 45, 46; 4 proteins in group c, protein 53, 54, 55, 56; 4 proteins in group d, protein 64, 65, 66, 67. There are also other proteins distributed over MZ-bell and MZ-leaf, amounted to 33 proteins.

Also in figure 18, MZ-mice gall tissues (G) were compared with MZ-leaf (A), there are 4 proteins in group a in MZ-mice, protein 26, 27, 28, 70. And in MZ-leaf, there are 4 proteins in group b, protein 43, 44, 45, 46; 4 proteins in group c, protein 53, 54, 55, 56; 4 proteins in group d, protein 64, 65, 66, 67. There are also other proteins distributed over MZ-mice and MZ-leaf, amounted to 28 proteins.

4.2.6.2 Protein pattern differences between bell-gall-tissues, mice-gall-tissues, and leaf tissues of *Machilus zuihoensis var. mushaensis.* (MM)

In figure 19, MM-bell gall tissues (E) compared with MM-leaf (B), there are 5 proteins in group a in MM-bell, protein 26, 27, 28, 70, 83. And in MM-leaf, there are 4 proteins in group b, protein 43, 44, 45, 46; 4 proteins in group c, protein 53, 54, 55, 56; 2 proteins in group d, protein 65, 66. There are also other proteins distributed over MM-bell and MM-leaf, amounted to 34 proteins.

Also in figure 19, MM-mice gall tissues (H) compared with MM-leaf (B), there are 5 proteins in group a in MM-mice, protein 26, 27, 28, 83, 88. And in MM-leaf, there are 4 proteins in group b, protein 43, 44, 45, 46; 4 proteins in group c, protein 53, 54, 55, 56; 2 proteins in group d, protein 65, 66. There are also other proteins distributed over MM-mice and MM-leaf, amounted to 25 proteins.

4.2.6.3 Protein pattern differences between bell-gall-tissues, mice-gall-tissues, and leaf tissues of *Machilus thunbergii.* (MT)

In figure 20, MT-bell gall tissues (F) compared with MT-leaf (C), there are 4 proteins in group a in MT-bell, protein 26, 27, 70, 93. And in MT-leaf, there are 4 proteins in group b, protein 43, 44, 45, 46; 5 proteins in group c, protein 53, 54, 55, 56, 98; 4 proteins in group d, protein 64, 65, 66, 67. There are also other proteins distributed over mt-bell and mt-leaf, amounted to 25 proteins.

Also in figure 20, MT-mice gall tissues (I) compared with MT-leaf (C), there are 4 proteins in group a in MT-bell, protein 26, 27, 70, 93. And in MT-leaf, there are 4 proteins in group b, protein 43, 44, 45, 46; 5 proteins in group c, protein 53, 54, 55, 56, 98; 4 proteins in group d, protein 64, 65, 66, 67. There are also other proteins distributed over mt-bell and mt-leaf, amounted to 30 proteins.

4.2.7 Mass Spectrometric sequencing of proteins

After analyzing and comparing all 2D images, we chose 33 outstanding proteins among all expressed proteins for in-gel digestion (figure 11). Then we sent these proteins to professor Chao-Hsiung Lin's laboratory in National Yang-Ming University for ESI-Q-TOF Mass Spectrometric analysis. The file type of protein amino sequences is .pkl, and we applied these sequences to MASCOT software (http://www.matrixscience.com) and tried to search similar protein sequences in the database of NCBInr. Ten proteins have high MASCOT scores, which represents more trustworthy results. These proteins are:

gi|3851512: RuBisCO large subunit

gi|57338572: RuBisCO large subunit

gi|37992949: RuBisCO large subunit

gi|18831: Mitochondrial ATP synthase beta-subunit

gi|3033513: Rubisco activase；

gi|19157: 33kDa precursor protein of oxygen-evolving complex；

gi|3914592: RuBisCO small subunit；

gi|9796478: Serine threonine kinase homolog COK-4

gi|7573598 : Protein kinase 2；

gi|24473812: Small ribosomal subunit protein 4。

The detailed information about these proteins is as appendix 8. Other proteins are not listed because of low MASCOT scores.

In these10 identified proteins, 7 proteins are largely reduced in gall tissues than in healthy leaf tissues. They are: 3 RuBisCO large subunit (gi|3851512, gi|57338572, gi|37992949); RuBisCO small subunit (gi|3914592); RuBisCO activase (gi|3033513); 33kDa precursor protein of oxygen-evolving complex (gi|19157); and serine threonine kinase homolog COI-4 (gi|18831). Other 3 proteins are expressed higher in both type of gall tissues in MT than in MM and MZ. These 3 proteins are: protein kinase 2 (gi|7573598); mitochondrial ATP synthase beta-subunit (gi|18831); and small ribosomal subunit protein 4 (gi|24473812).

Besides serine threonine kinase homolog COK-4, proteins which were reduced in gall tissues are directly related to plant photosynthesis. It indicates that photosynthesis function might be inefficient in gall tissues.

In fig 21 proteins in group b are RuBisCO large subunit, and proteins in group d are RuBisCO small subunit. Proteins in both groups are largely reduced in gall tissues.

Ribulose-1,5-bisphosphate carboxylase/oxygenase, also known as RuBisCO, is an enzyme (EC 4.1.1.39) that is used in the Calvin cycle to catalyze the first major step of carbon fixation, a process by which the atoms of atmospheric carbon dioxide are made available to organisms in the form of energy-rich molecules such as sucrose. RuBisCO catalyzes either the carboxylation or oxygenation of ribulose-1,5-bisphosphate (also known as RuBP) with carbon dioxide or oxygen.

In plants, algae, cyanobacteria, and phototropic and chemoautotropic proteobacteria the enzyme usually consists of two types of protein subunit, called the large chain (L, about 55,000 Da) and the small chain (S, about 13,000 Da). The enzymatically active substrate (ribulose 1,5-bisphosphate) binding sites are located in the large chains that form dimmers, in which amino acids from each large chain contribute to the binding sites.

The reduced expression of RuBisCO in gall tissues suggests that the photosynthesis function in galls is inefficient. In botany, leaf is classified as nutrition tissue because of the nutrition generated from photosynthesis. Since gall tissues loss their photosynthesis function, we observed that *Machilus* with large amount of galls grows worse than *Machilus* without or with less galls.

Protein number 103, serine threonine kinase homolog COK-4 is also reduced in gall tissues. Serine/threonine kinases all phosphorylate serine or threonine residues in their substrates. A kinase is usually not specific to a single substrate, but instead can phosphorylate a whole "substrate family" having common recognition sequences. Activity of these protein kinases can be regulated by specific events (e.g. DNA damage), as well as numerous chemical signals, including: cAMP/cGMP, diacylglycerol, Ca^{2+}/camodulin…etc. There are too many possibilities of this protein, therefore we don't know about its functions in gall tissues.

There are 3 proteins expressed obviously higher in both type of MT gall tissues than in MM and MZ: protein kinase 2 (gi|7573598); mitochondrial ATP synthase beta-subunit (gi|18831); and small ribosomal subunit protein 4 (gi|24473812). This might indicate that some physiological reactions inside MT gall tissues are different form that of MZ and MM.

Besides aforementioned proteins, other proteins which have lower MOSCOT scores are mostly lipid-binding proteins and protein kinase. Phospholipid-binding proteins are an important component of cellular signalling, trafficking, and metabolism. Nonspecific lipid transfer proteins (nsLTPs) facilitate the transfer of phospholipids, glycolipids, fatty acids and steroids between membranes, with wide-ranging binding affinities. Because of their important role in physiology, it's normal to have many lipid proteins identified.

4.3 Combined discussion of gall midges phylogeny and plant proteomics.

In past researches, entomologists suppose that Cecidomyiidae midges which made galls on same *Machilus* species are same species because of their host specificity.

In our experiment, gall midges which make same types of galls are closer species, and they induce galls on different *Machilus* species. We suppose that same morphospecies secret same chemicals to different *Machilus* and induce same types of galls. *Machilus* reacts with these chemicals, be induced same type of galls, but have different resulted proteins. Since chemicals which midge secretes are supposed the same, the protein difference came from plant difference.

The evidence is, in plant classification, MZ and MM are very similar in many traits, some botanists regard them as different species but some don't (自然保育季刊49：31-35，,March, 2005), and they are definitely different species with MT. And in our analysis, the mice morphospecies are all same species, and the proteins differences between MZ-mice & MM-mice are smaller than the difference between MZ-mice & MT-mice or MM-mice & MT-mice. This situation happened in bell gall tissues on MZ, MM, and MT as well.

At the other hand, midges which make different types of galls are different species (even genus or tribes.). We suppose that they secret different chemicals and induce different types of galls on *Machilus*. We compared the protein patterns between bell and mice gall tissues on same *Machilus* species, and there are only slight protein differences. These proteins might play an important role on gall-type-forming, but we're not sure whether these proteins are inducers or resultants.

The evidence is, we compared bell & mice galls on each *Machilus* separately (Fig 15). The amount of different proteins of bell & mice galls on MZ and MM are similar (6 and 5 proteins each), but there are only 3 different proteins between MT-mice and MT-bell. When we compare the shape of MT-bell galls with other bell galls and MT-mice galls, we can clearly see that MT-bell galls are different from other bell galls. Unlike other bell galls, there are no crest lines on MT-bell galls, and MT-bell galls are as round as MT-mice galls. The shape of MT-bell galls are between typical bell galls and mice galls, and the protein difference are smaller between MT-bell and MT-mice. Therefore, we believe that these proteins might play an important role on gall formation, but we can't be sure whether they are inducers or resultants.

Sum up aforementioned two conclusions, we have the third conclusion. Protein differences between bell & mice galls on same *Machilus* are smaller than the protein differences between same galls on different *Machilus*. It might because different midges species secret similar chemicals to reduce different galls. And different *Machilus* species react differently to same chemicals.

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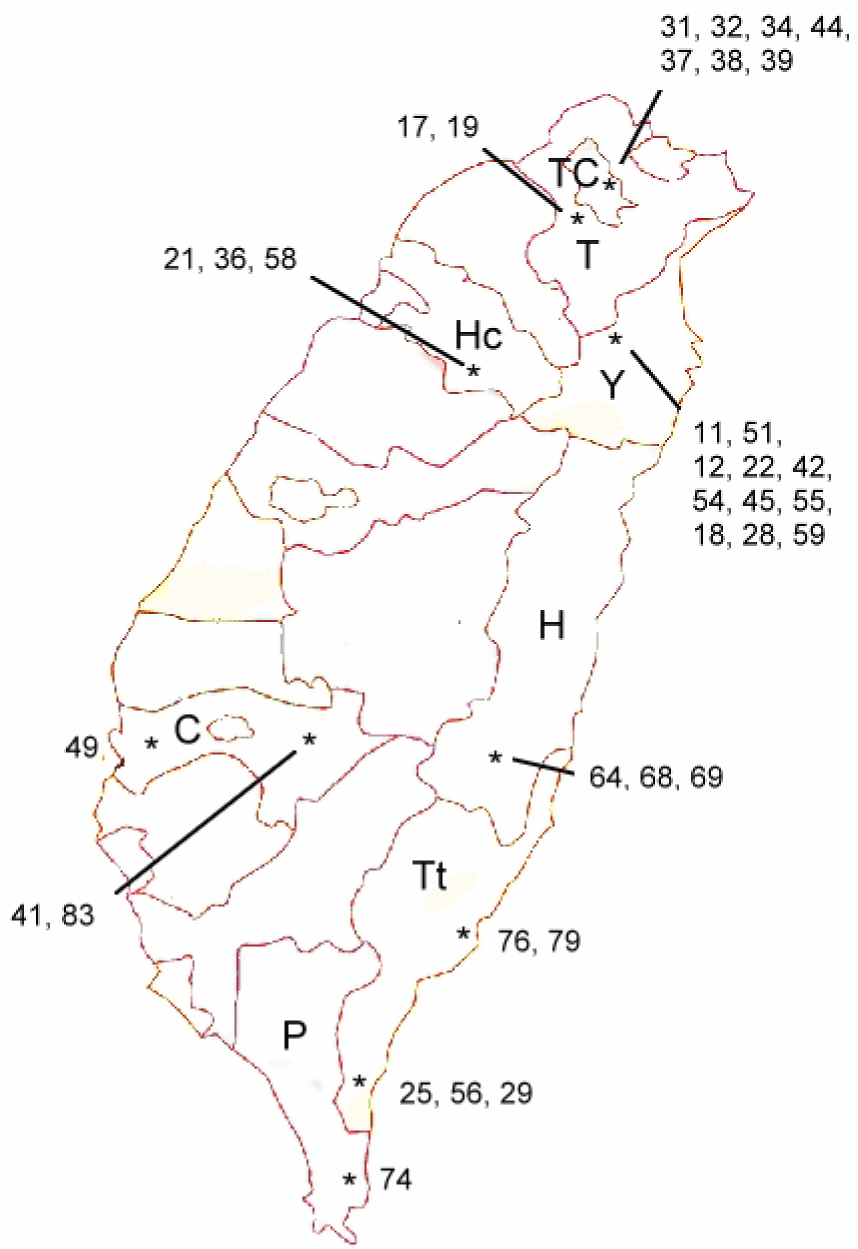
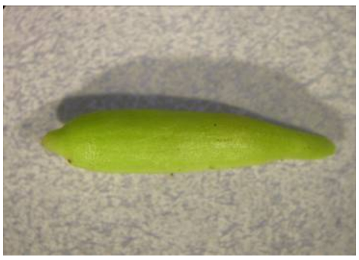
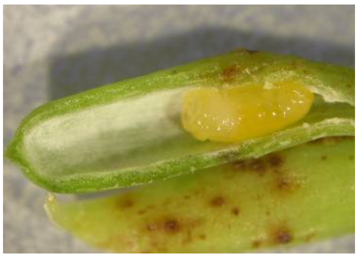
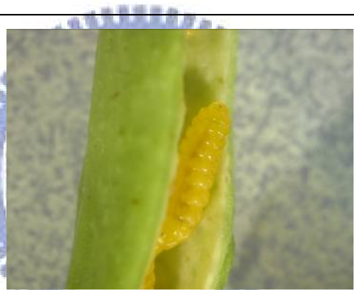


Figure. 1. Sample locations of cecidomyiidae gall midege in Taiwan .

TC: Taipei city; T: Taipei county Hc:Hsin-chu county; Y: Yilan county; H: Hualian county; Tt: Taitung county; P: Pintung county; C: Chayi county. The numbers behind the toponyms are the serial number in Table 1 and Table 2.

		
mt-mice	mt-mice	mt-mice
		
mm-bell	mm-bell	mm-bell
		
mt-bird	mt-bird	mt-bird
		
mt-club	mt-club	mt-club

		
mm-bulb	mm-bulb	mm-bulb
		
mt-blister (Dr. Tung)	mt-blister (Dr. Tung)	mt-blister (Dr. Tung)
		
mz-bud (long)	mz-bud (long)	mz-bud (long)
		
mz-bud (round)	mz-bud (round)	mz-bud (round)
		

mz-bullet	mjk-bullet (Dr. Tung)	mz-bullet
		
mz-spindle (Dr. Tung)	mm-spindle (Dr. Tung)	mz-spindle (Dr. Tung)

Figure 2. Cecidomyiidae midge larvae and galls

We named each sample according to plant species and gall forms. There are 9 different types of galls and eight plant species. These 9 different types of galls are: mice, bell, bird, club, bulb, bullet, bud, spindle, and blister. From each type of galls, we choose one gall picture and two larvae pictures. Noted pictures were provided by Dr. Tung in TFRI.

	
mjk-bell	mjk-bell
	
mj-mice	mj-mice
	
mjk-bell	mjk-bell

	
mt-bell	mj-club
	
mz-bell	mj-mice
	
mjk-bell	mjk-bell

	
mj-blister	mj-blister

Figure 3. Parasitized midge larvae

Cecidomyiidae midge larvae are easily be parasitized by bees or other organisms such as fungi, bacteria…etc. When parasites were inside the larvae, we abandoned these larvae. When parasites were outside the larvae, we picked parasites out from midge larvae. Then we put these once-parasitized larvae into another container, separated from those un-parasitized larvae in order to avoid contamination. We would use these once-parasitized larvae only when we ran out of un-parasitized larvae. In our experiment, we didn't use these once-parasitized larvae.

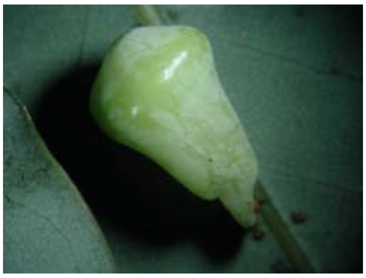
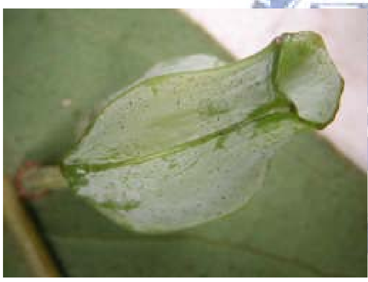
	
MT bell	**MT mice**
	
MM bell	**MM mice**
	
MZ bell	**MZ mice**

Figure 4. Plant samples

MT: *Machilus thunbergii*, **MM**: *Machilus zuihoensis var. mushaensis*, **MZ**: *Machilus zuihoensis var. zuihoensis*, **bell:** bell shaped gall. **mice**: mice shaped gall.

Plant samples were collected and photographed by Hung-Pin Chen.

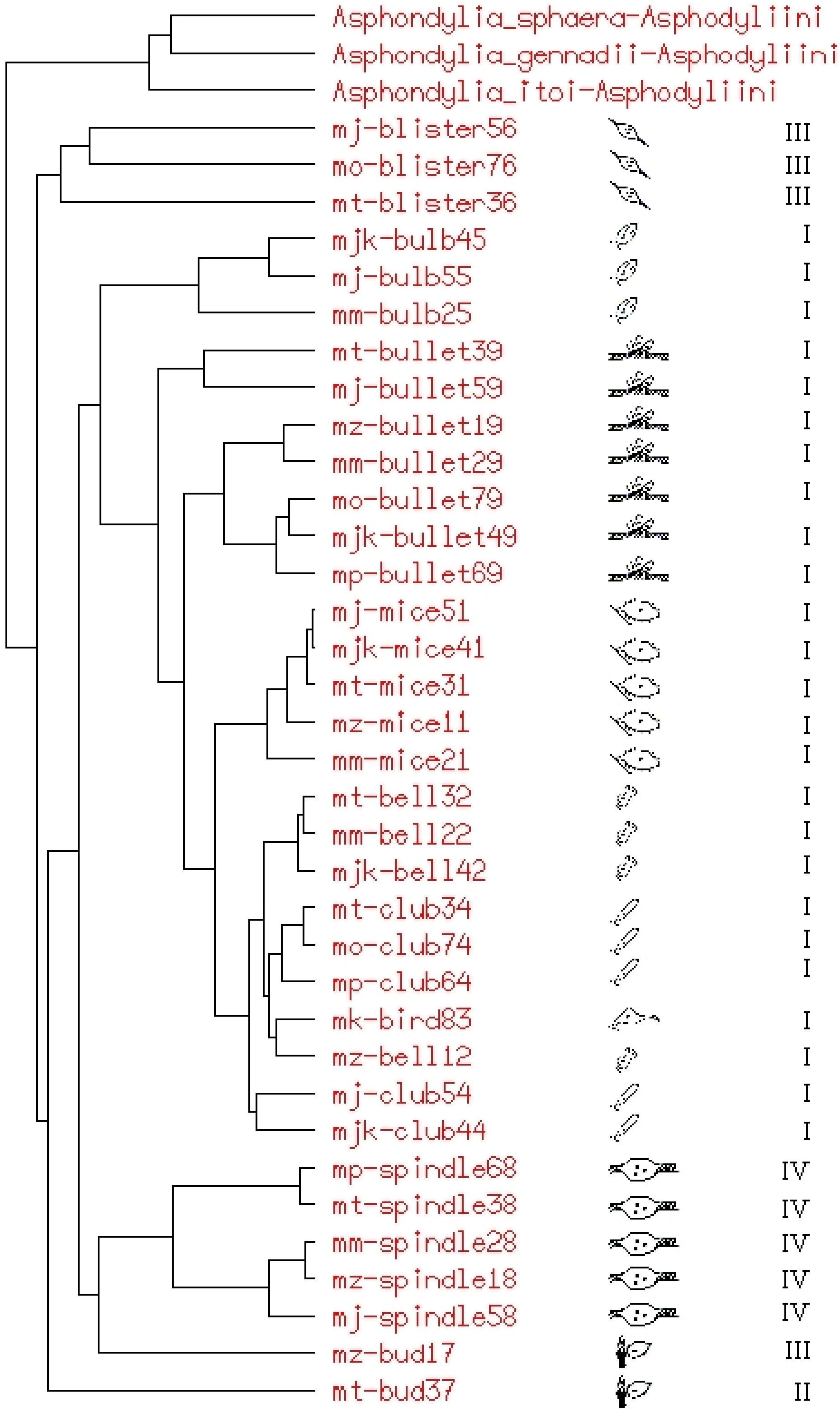

Asphondylia_sphaera-Asphodyliini
Asphondylia_gennadii-Asphodyliini
Asphondylia_itoi-Asphodyliini
mj-blister56 III
mo-blister76 III
mt-blister36 III
mjk-bulb45 I
mj-bulb55 I
mm-bulb25 I
mt-bullet39 I
mj-bullet59 I
mz-bullet19 I
mm-bullet29 I
mo-bullet79 I
mjk-bullet49 I
mp-bullet69 I
mj-mice51 I
mjk-mice41 I
mt-mice31 I
mz-mice11 I
mm-mice21 I
mt-bell32 I
mm-bell22 I
mjk-bell42 I
mt-club34 I
mo-club74 I
mp-club64 I
mk-bird83 I
mz-bell12 I
mj-club54 I
mjk-club44 I
mp-spindle68 IV
mt-spindle38 IV
mm-spindle28 IV
mz-spindle18 IV
mj-spindle58 IV
mz-bud17 III
mt-bud37 II

Figure 5. Cecidomyiidae COI gene sequence alignment. There are 3 out-group sequences and 35 sample sequences. Unknown gall midge species were named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. **1. MZ:** *Machilus zuihensis Hayata.* **2. MM***:* *Machilus zuihoensis. var. mushaensis.* **3. MT**: *Machilus thunbergii.* **4. MJK:** *Machilus japonica kusanoi.* **5. MJ:** *Machilus japonica.* **6. MP:** *Machilus philippinense.* **7. MO:** *Machilus obovatifolia.* ***8.*** **MK**: *Machilus konishii Hayata.* Letters after dash line are gall types.1.mice. 2. bell. 3. bird. 4. club. 5. bulb. 6. blister. 7. bud. 8. spindle. 9. bullet. Numbers beside sketches are larva types in table 5.

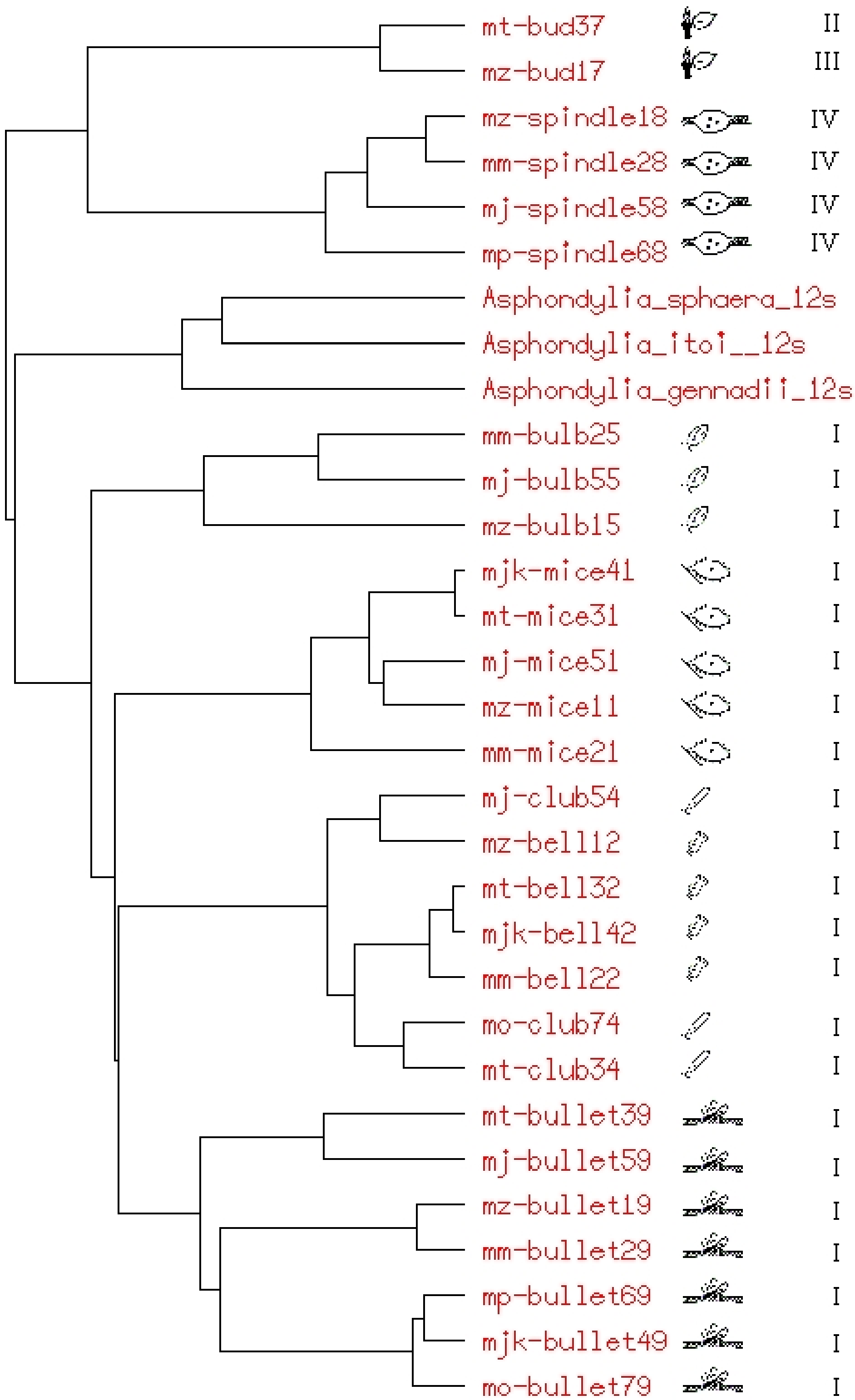
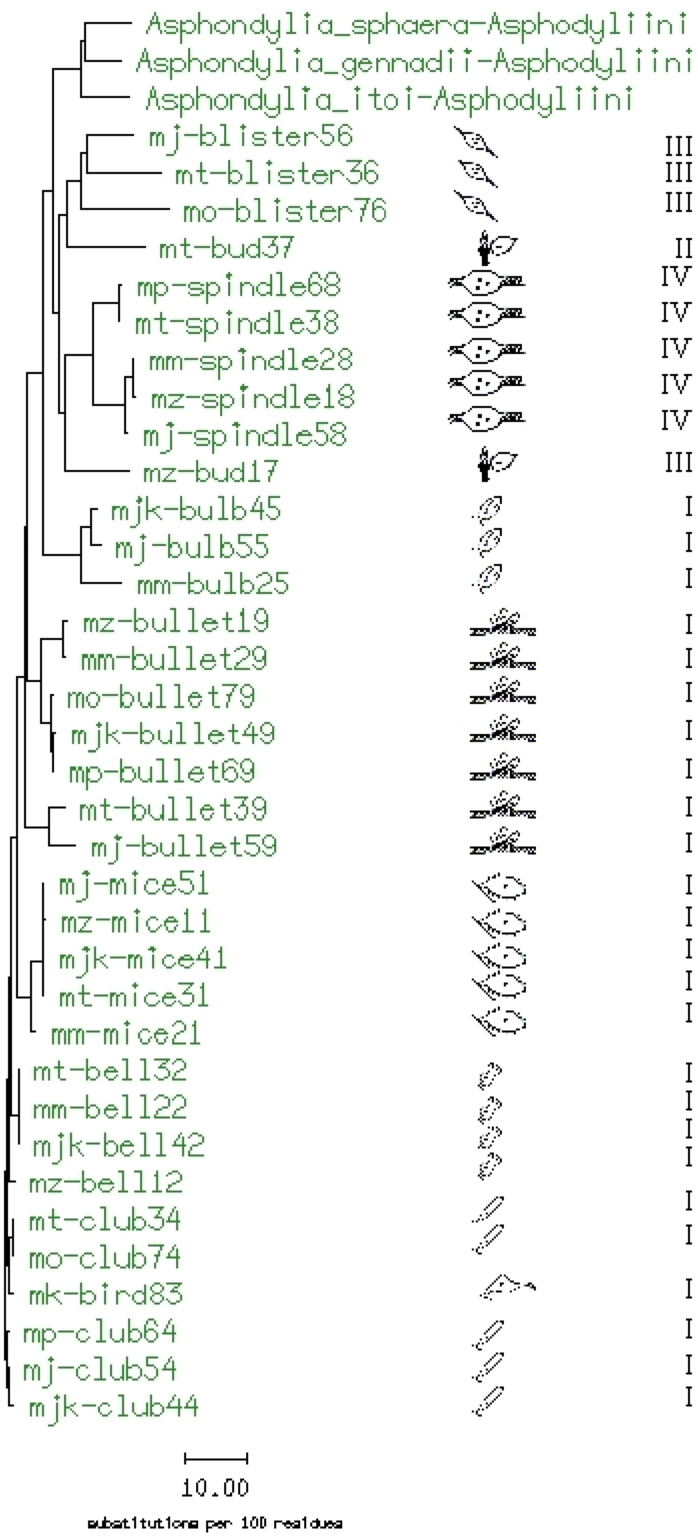

mt-bud37 II
mz-bud17 III
mz-spindle18 IV
mm-spindle28 IV
mj-spindle58 IV
mp-spindle68 IV
Asphondylia_sphaera_12s
Asphondylia_itoi__12s
Asphondylia_gennadii_12s
mm-bulb25 I
mj-bulb55 I
mz-bulb15 I
mjk-mice41 I
mt-mice31 I
mj-mice51 I
mz-mice11 I
mm-mice21 I
mj-club54 I
mz-bell12 I
mt-bell32 I
mjk-bell42 I
mm-bell22 I
mo-club74 I
mt-club34 I
mt-bullet39 I
mj-bullet59 I
mz-bullet19 I
mm-bullet29 I
mp-bullet69 I
mjk-bullet49 I
mo-bullet79 I

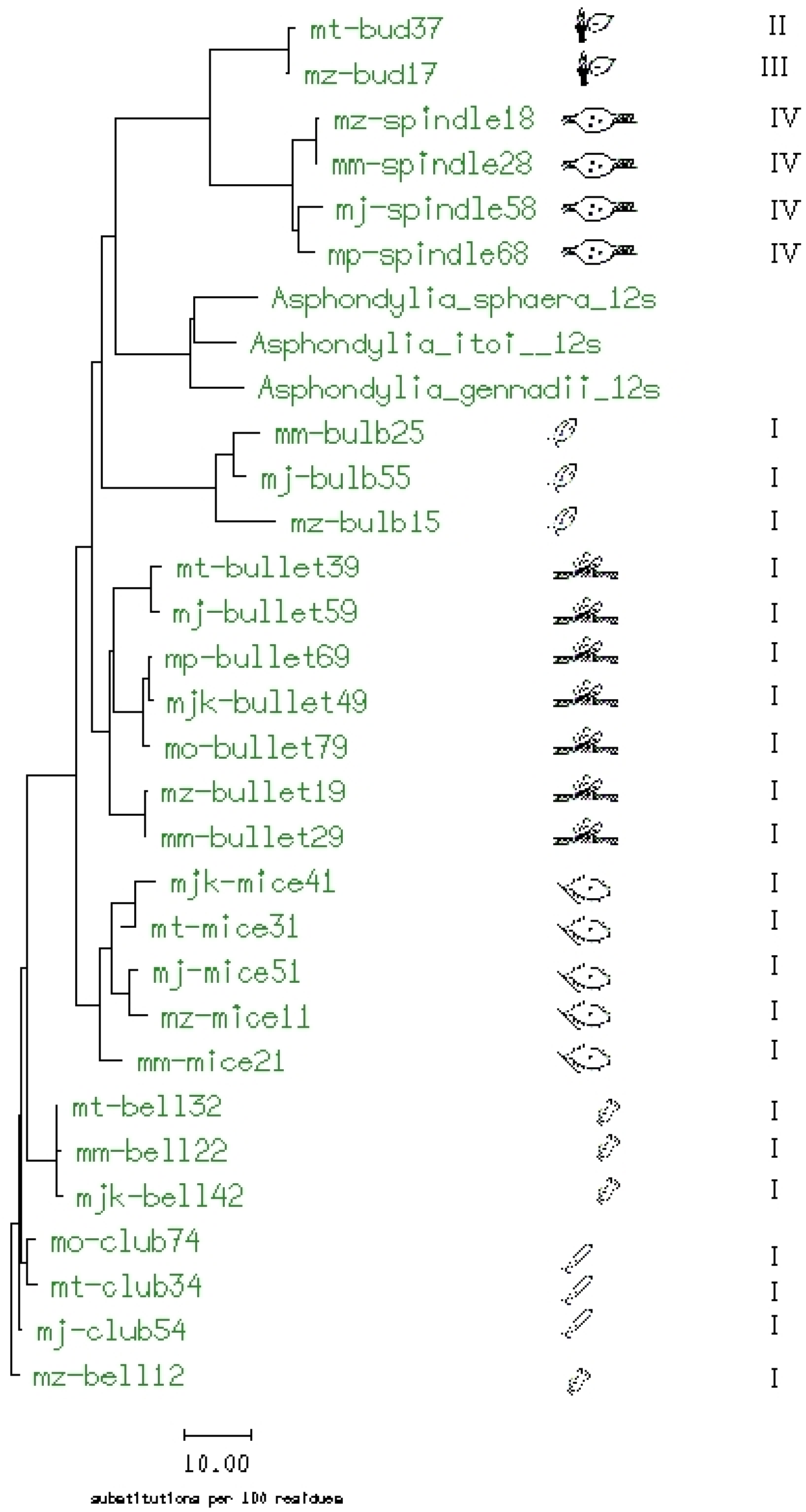
Figure 6. Cecidomyiidae 12s gene sequence alignment. Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2 Letters before dash line are host plane abbreviation. **1.MZ :** *Machilus zuihensis Hayata.* **2.MM:** *Machilus zuihoensis. var. mushaensis.* **3.MT**: *Machilus thunbergii.* **4.MJK:** *Machilus japonica kusanoi.* **5.MJ:** *Machilus japonica.* **6.MP:** *Machilus philippinense.* **7.MO:** *Machilus obovatifolia.* ***8.*****MK**: *Machilus konishii Hayata.* Letters after dash line are gall types.1.mice. 2.bell. 3.bird. 4.club. 5.bulb. 6.blister. 7.bud. 8.spindle. 9.bullet. Numbers beside sketches are larva types in table 5.



Asphondylia_sphaera-Asphodyliini
Asphondylia_gennadii-Asphodyliini
Asphondylia_itoi-Asphodyliini
mj-blister56 III
mt-blister36 III
mo-blister76 III
mt-bud37 II
mp-spindle68 IV
mt-spindle38 IV
mm-spindle28 IV
mz-spindle18 IV
mj-spindle58 IV
mz-bud17 III
mjk-bulb45 I
mj-bulb55 I
mm-bulb25 I
mz-bullet19 I
mm-bullet29 I
mo-bullet79 I
mjk-bullet49 I
mp-bullet69 I
mt-bullet39 I
mj-bullet59 I
mj-mice51 I
mz-mice11 I
mjk-mice41 I
mt-mice31 I
mm-mice21 I
mt-bell32 I
mm-bell22 I
mjk-bell42 I
mz-bell12 I
mt-club34 I
mo-club74 I
mk-bird83 I
mp-club64 I
mj-club54 I
mjk-club44 I
10.00
substitutions per 100 residues

Figure 7. Evolutionary tree of gall midge COI DNA with Neighbor-Joining method.

This neighbor-joining tree for gall-midges of *Machilus* is based on average 396 bp of the mitochondrial COI gene and Kimura's two-parameter distance. There are 3 out-group sequences and 35 sample sequences. Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. **1. MZ :** *Machilus zuihensis Hayata.* **2. MM:** *Machilus zuihoensis. var. mushaensis.* **3. MT**: *Machilus thunbergii.* **4. MJK:** *Machilus japonica kusanoi.* **5. MJ:** *Machilus japonica.* **6. MP:** *Machilus philippinense.* **7. MO:** *Machilus obovatifolia.* ***8.*** **MK**: *Machilus konishii Hayata.* Letters after dashed line are gall types.1.mice. 2. bell. 3. bird. 4. club. 5. bulb. 6. blister. 7. bud. 8. spindle. 9. bullet. The dots in each sketch represent how many larvae are in one gall. One dot means there is only one larva in one gall. Three dots represent that there are more than one larva in one gall. Numbers beside sketches are larva types in table 4.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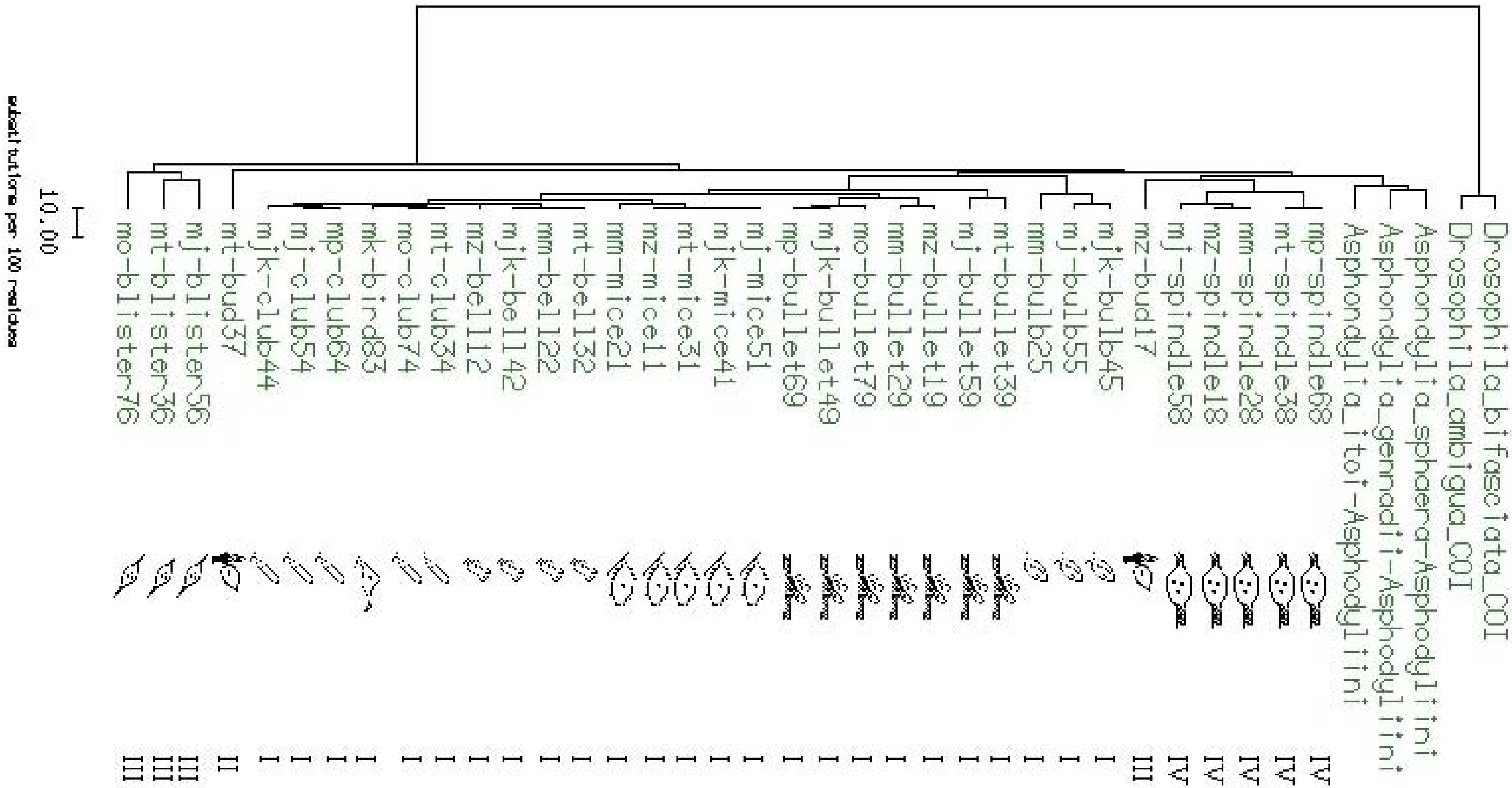
mz-bud17 III
mz-spindle18 IV
mm-spindle28 IV
mj-spindle58 IV
mp-spindle68 IV
Asphondylia_sphaera_12s
Asphondylia_itoi__12s
Asphondylia_gennadii_12s
mm-bulb25 I
mj-bulb55 I
mz-bulb15 I
mt-bullet39 I
mj-bullet59 I
mp-bullet69 I
mjk-bullet49 I
mo-bullet79 I
mz-bullet19 I
mm-bullet29 I
mjk-mice41 I
mt-mice31 I
mj-mice51 I
mz-mice11 I
mm-mice21 I
mt-bell32 I
mm-bell22 I
mjk-bell42 I
mo-club74 I
mt-club34 I
mj-club54 I
mz-bell12 I
10.00
substitutions per 100 residues

Figure 8. Evolutionary tree of gall midge 12S DNA with Neighbor-Joining method.

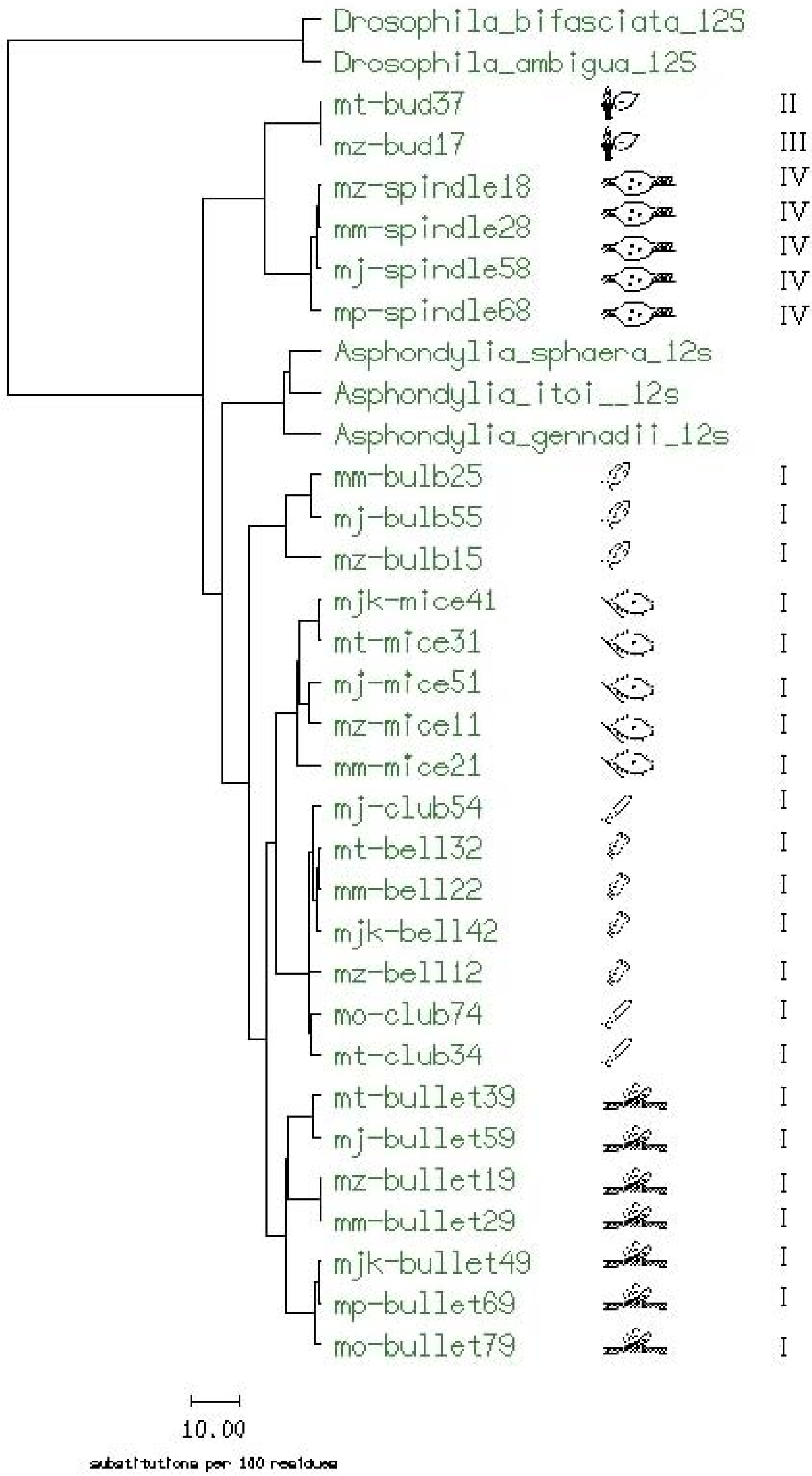
This neighbor-joining tree for gall-midges of *Machilus* is based on average 430 bp of the mitochondrial 12s gene and Kimura's two-parameter distance. Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. **1.MZ :** *Machilus zuihensis Hayata.* **2.MM*:*** *Machilus zuihoensis. var. mushaensis.* **3.MT**: *Machilus thunbergii.* **4.MJK:** *Machilus japonica kusanoi.* **5.MJ:** *Machilus japonica.* **6.MP:** *Machilus philippinense.* **7.MO:** *Machilus obovatifolia.* ***8.*MK**: *Machilus konishii Hayata.* Letters after dash line are gall types.1.mice. 2.bell. 3.bird. 4.club. 5.bulb. 6.blister. 7.bud. 8.spindle. 9.bullet. The dots in each sketch represent how many larvae are in one gall. One dot means there is only one larva in one gall. Three dots represent that there are more than one larva in one gall. Numbers beside sketches are larva types in table 5.



Drosophila_bifasciata_COI
Drosophila_ambigua_COI
Asphondylia_sphaera-Asphondyliini
Asphondylia_gennadii-Asphondyliini
Asphondylia_itoi-Asphondyliini
mp-spindle68 IV
mt-spindle38 IV
mm-spindle28 IV
mz-spindle18 IV
mj-spindle58 IV
mz-bud17 III
mjk-bulb45 I
mj-bulb55 I
mm-bulb25 I
mt-bullet39 I
mj-bullet59 I
mz-bullet19 I
mm-bullet29 I
mo-bullet79 I
mjk-bullet49 I
mp-bullet69 I
mj-mice51 I
mjk-mice41 I
mt-mice31 I
mz-mice11 I
mm-mice21 I
mt-bell132 I
mm-bell122 I
mjk-bell142 I
mz-bell112 I
mt-club34 I
mo-club74 I
mk-bird83 I
mp-club64 I
mj-club54 I
mjk-club44 I
mt-bud37 II
mj-blister56 III
mt-blister36 III
mo-blister76 III
10.00
substitutions per 100 residues

Figure 9. Evolutionary tree of gall midge COI gene using UPGMA method.

This UPGMA tree for gall-midges of *Machilus* is based on average 396 bp of the mitochondrial COI gene and Kimura's two-parameter distance. Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. **1.MZ :** *Machilus zuihensis Hayata.* **2.MM:** *Machilus zuihoensis. var. mushaensis.* **3.MT**: *Machilus thunbergii.* **4.MJK:** *Machilus japonica kusanoi.* **5.MJ:** *Machilus japonica.* **6.MP:** *Machilus philippinense.* **7.MO:** *Machilus obovatifolia.* ***8.*****MK**: *Machilus konishii Hayata.* Letters after dash line are gall types.1.mice. 2.bell. 3.bird. 4.club. 5.bulb. 6.blister. 7.bud. 8.spindle. 9.bullet. The dots in each sketch represent how many larvae are in one gall. One dot means there is only one larva in one gall. Three dots represent that there are more than one larva in one gall. Numbers beside sketches are larva types in table 4.



Drosophila_bifasciata_12S
Drosophila_ambigua_12S
mt-bud37 II
mz-bud17 III
mz-spindle18 IV
mm-spindle28 IV
mj-spindle58 IV
mp-spindle68 IV
Asphondylia_sphaera_12s
Asphondylia_itoi__12s
Asphondylia_gennadii_12s
mm-bulb25 I
mj-bulb55 I
mz-bulb15 I
mjk-mice41 I
mt-mice31 I
mj-mice51 I
mz-mice11 I
mm-mice21 I
mj-club54 I
mt-bell32 I
mm-bell22 I
mjk-bell42 I
mz-bell12 I
mo-club74 I
mt-club34 I
mt-bullet39 I
mj-bullet59 I
mz-bullet19 I
mm-bullet29 I
mjk-bullet49 I
mp-bullet69 I
mo-bullet79 I
10.00
substitutions per 100 residues

Figure 10. Evolutionary tree of gall midge 12S gene using UPGMA method.

This UPGMA tree for gall-midges of *Machilus* is based on average 430 bp of the mitochondrial 12s gene and Kimura's two-parameter distance. Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. **1.MZ :** *Machilus zuihensis Hayata.* **2.MM***:* *Machilus zuihoensis. var. mushaensis.* **3.MT**: *Machilus thunbergii.* **4.MJK:** *Machilus japonica kusanoi.* **5.MJ:** *Machilus japonica.* **6.MP:** *Machilus philippinense.* **7.MO:** *Machilus obovatifolia.* ***8.*****MK**: *Machilus konishii Hayata.* Letters after dash line are gall types.1.mice. 2.bell. 3.bird. 4.club. 5.bulb. 6.blister. 7.bud. 8.spindle. 9.bullet. The dots in each sketch represent how many larvae are in one gall. One dot means there is only one larva in one gall. Three dots represent that there are more than one larva in one gall. Numbers beside sketches are larva types in table 4.

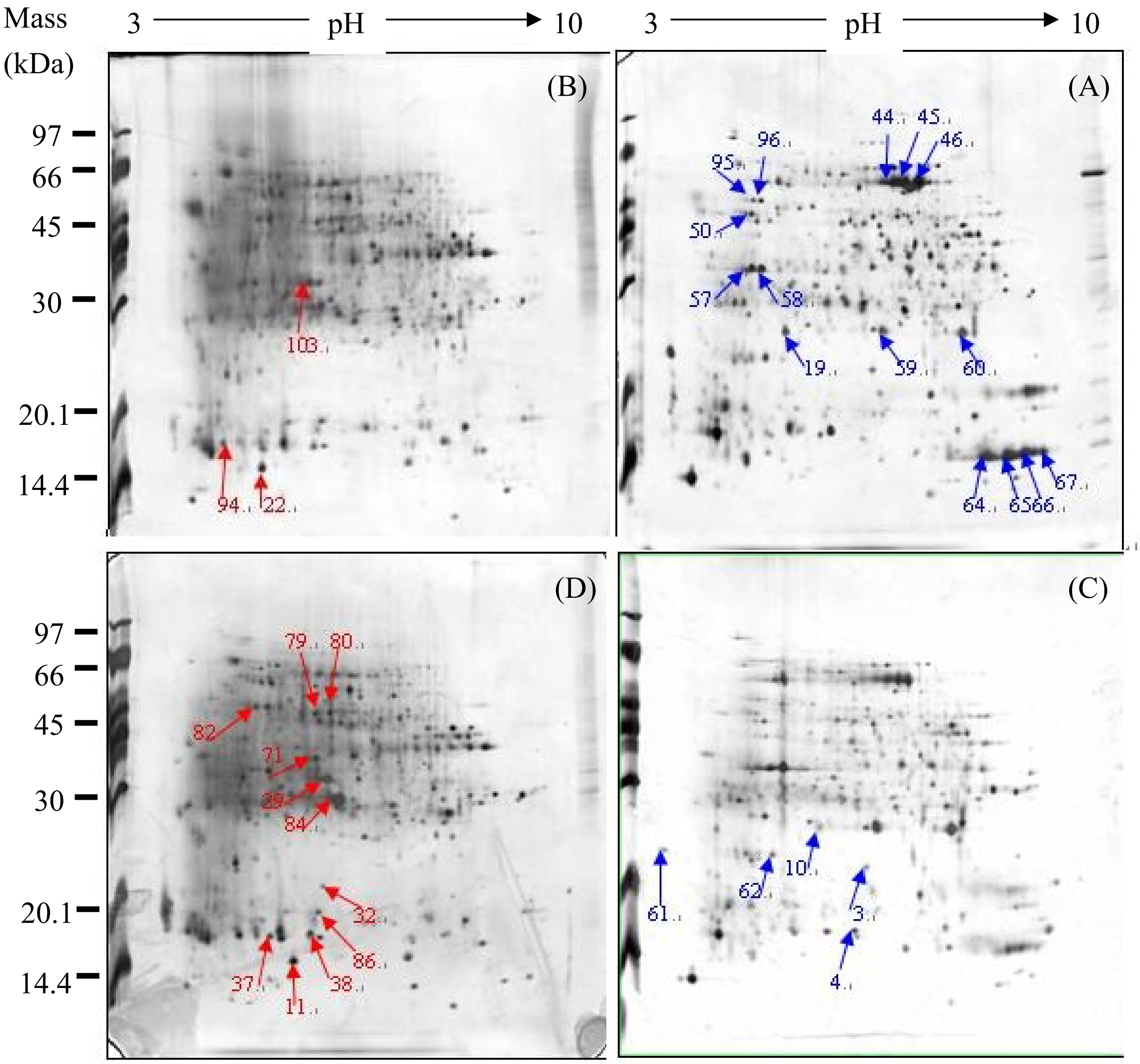


Figure 11. Protein spots which were sent to Mass Spectrometric Analysis

(A) MT-leaf: leaf tissues of *Machilus thunbergii*

(B) MT-bell: bell galls of *Machilus thunbergii.*

(C) MM-leaf: leaf tissues of *Machilus zuihoensis var. mushaensis.*

(D) MM-bell: bell galls of *Machilus zuihoensis var. mushaensis..*

34 labeled protein spots were sent to NYMU for Mass Spectrometric analysis.

Note: Experiment was done by Hung-Pin Chen. Data collection and arrangement were done by me.

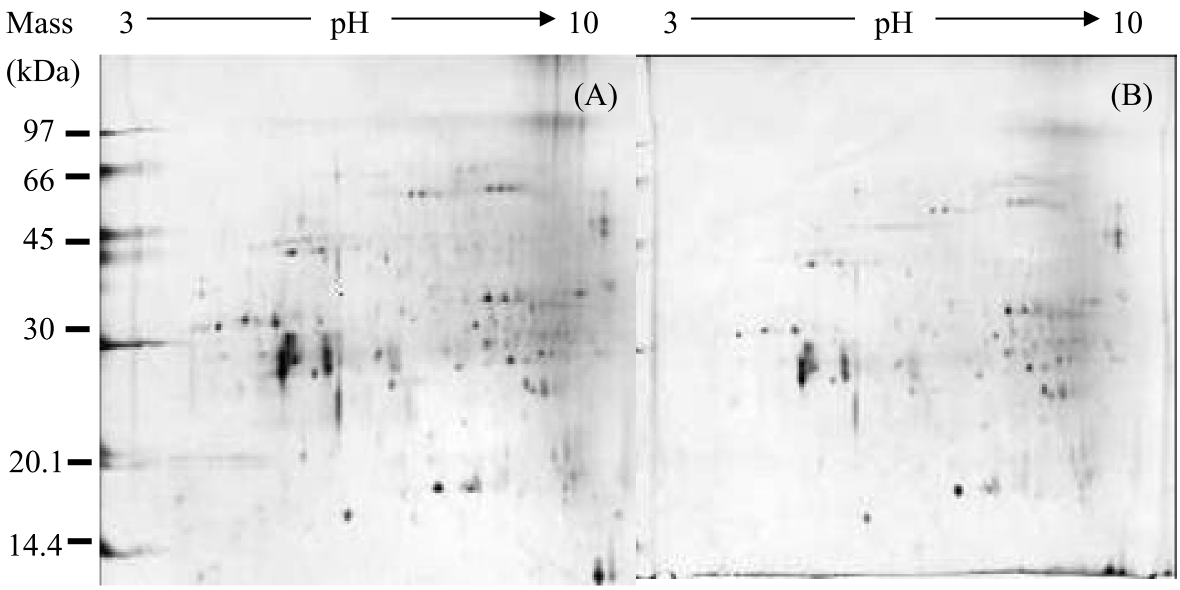
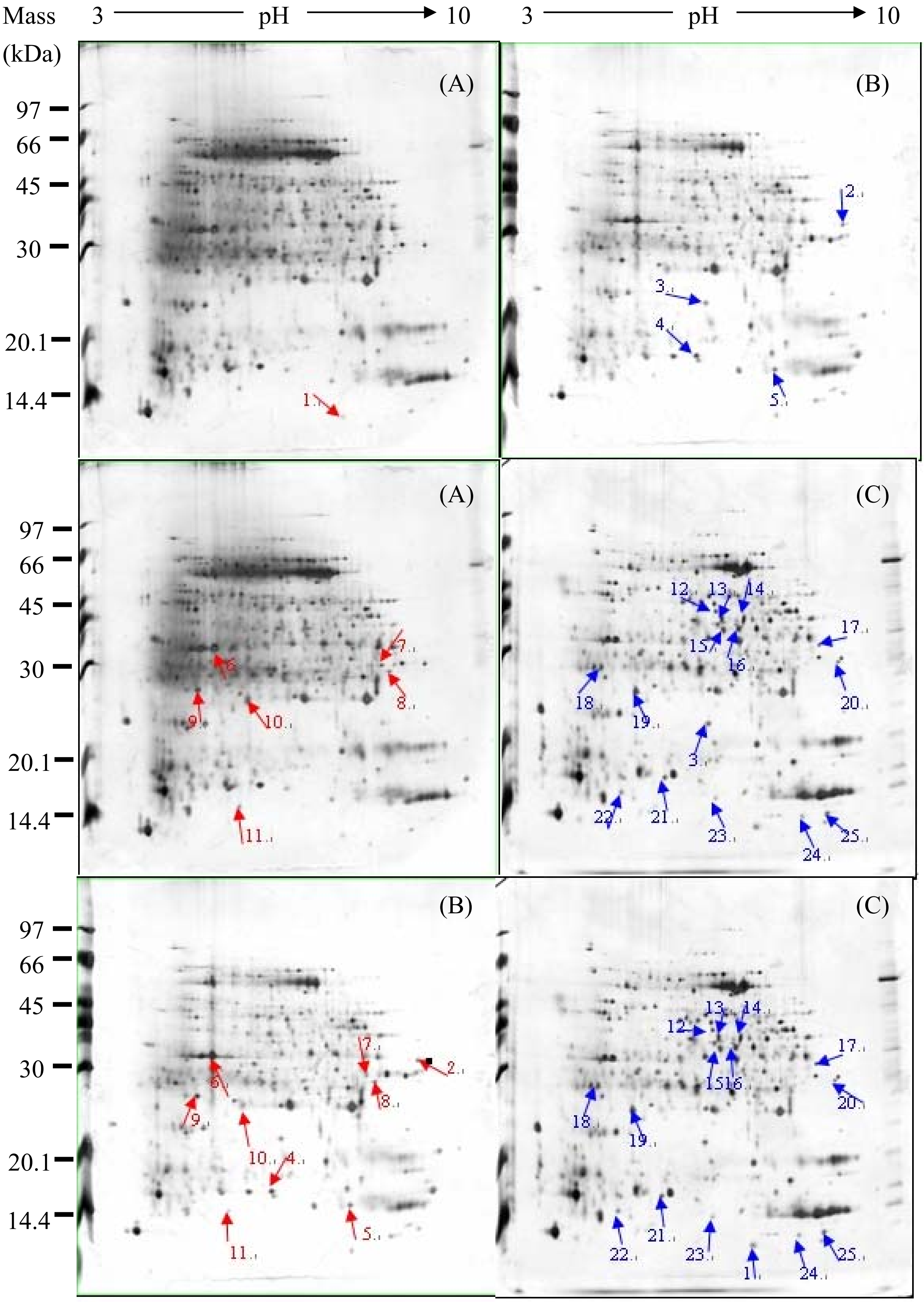


Figure 12. Protein patterns of galled and ungalled ***Machilus zuihoensis var. mushaensis*** **leaves.**

(A) Healthy leaf tissues which has gall grown on the same leaf.

(B) Healthy leaf tissues which has no gall grown on the surface.

Note: Experiment was done by Hung-Pin Chen. Data collection and arrangement were done by me.



Mass (kDa)
3 pH 10
(A)
(B)
(C)
97
66
45
30
20.1
14.4
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

Figure 13. Protein pattern differences between three *Machilus* leaves.

These three *Machilus* leaves were classified into three groups, (A) and (B), (B) and (C), (A) and (C). The leave protein patterns were compared with each other.

(A) MZ-leaf: leaf tissues of *Machilus zuihoensis var. zuihoensis*. **(B)** MM-leaf: leaf tissues of *Machilus zuihoensis var. mushaensis.* **(C)** MT-leaf: leaf tissues of *Machilus thunbergii.* Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

Note: Experiment was done by Hung-Pin Chen. Data collection and arrangement were done by me.

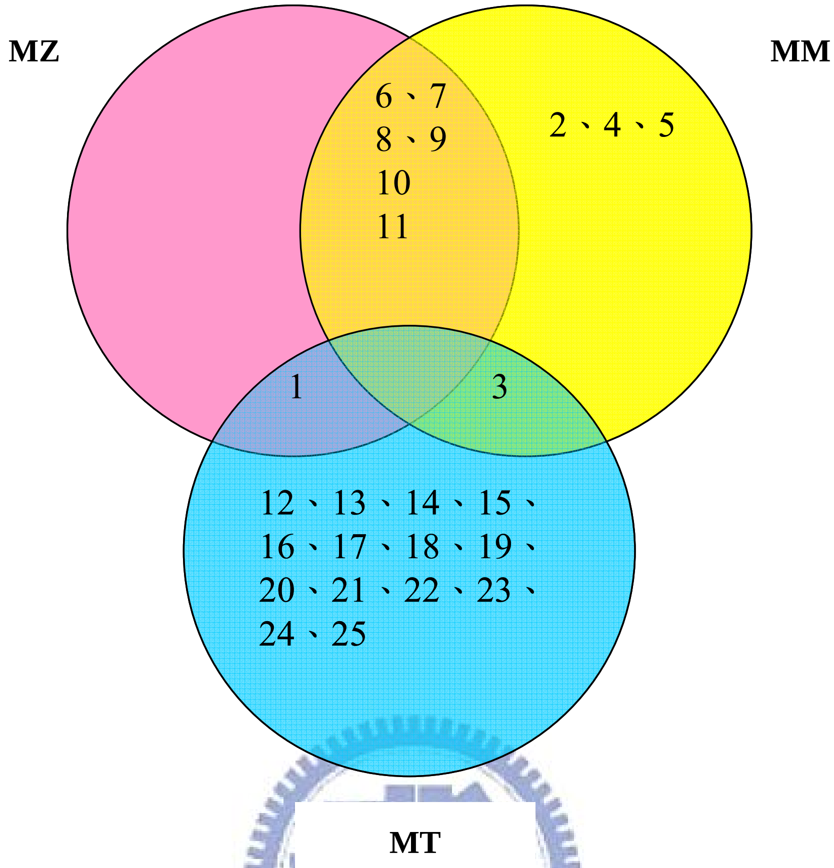


Figure 14. Protein pattern differences between three *Machilus* leaves.

MZ: *Machilus zuihoensis var. zuihoensis*. **MM**: *Machilus zuihoensis var. mushaensis*. **MT:** *Machilus thunbergii*. Numbers are protein spots labeled in **Fig 2.**

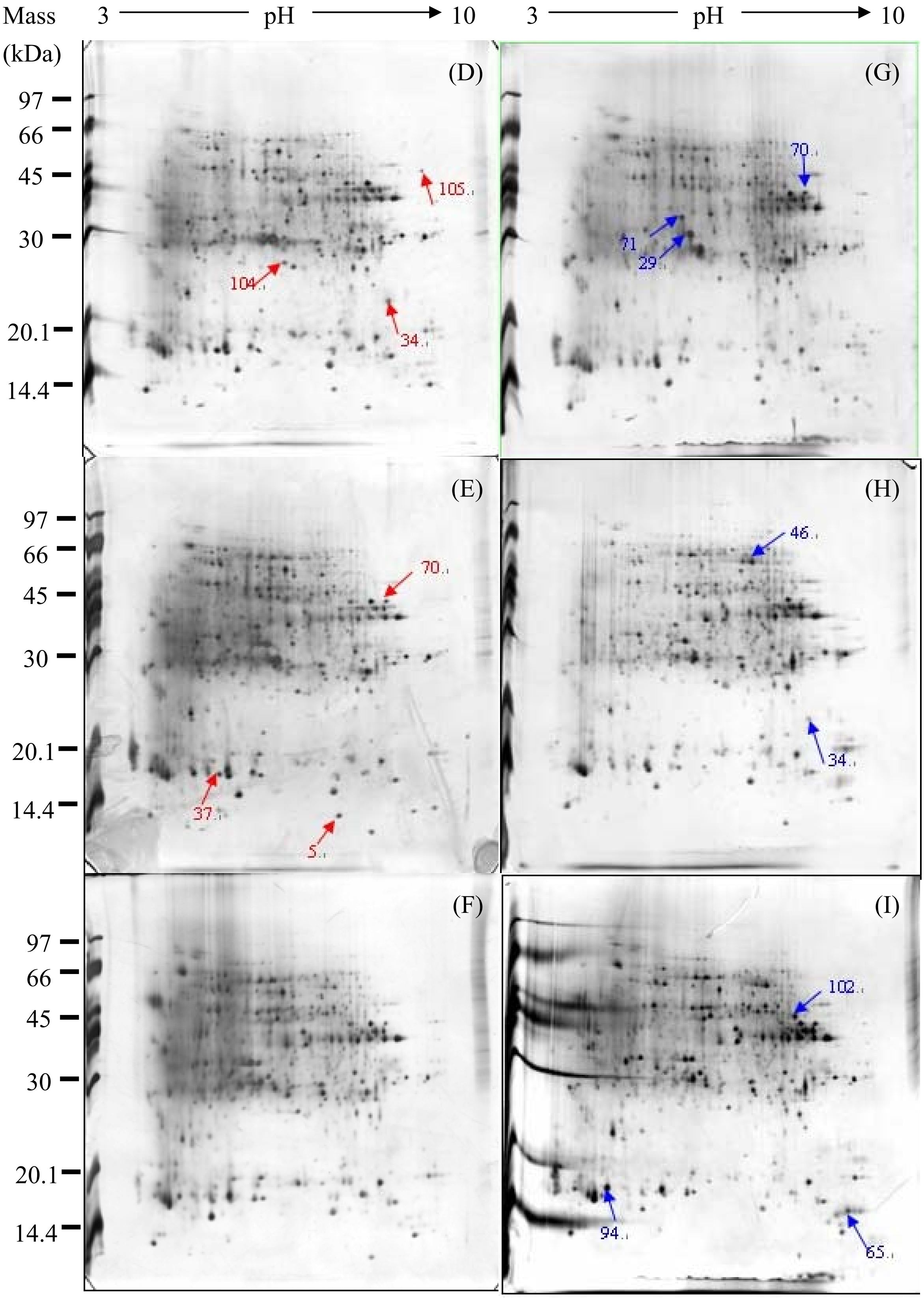

Mass (kDa)
3 pH 10
3 pH 10
97
66
45
30
20.1
14.4
(D)
105
104
34
(G)
70
71
29
(E)
70
37
5
(H)
46
34
(F)
(I)
102
94
65

Figure 15.Protein pattern differences of two types of galls on same *Machilus* species.

The samples are classified into three groups according to three *Machilus* species: (D) and (G), (E) and (H), (F) and (I). There are two types of galls in each group, bell gall and mice gall.

(D) MZ-bell: bell-gall tissues of *Machilus zuihoensis var. zuihoensis*.

(E) MM-bell: bell-gall tissues of *Machilus zuihoensis var. mushaensis.*

(F) MT-bell: bell-gall tissues of *Machilus thunbergii.*

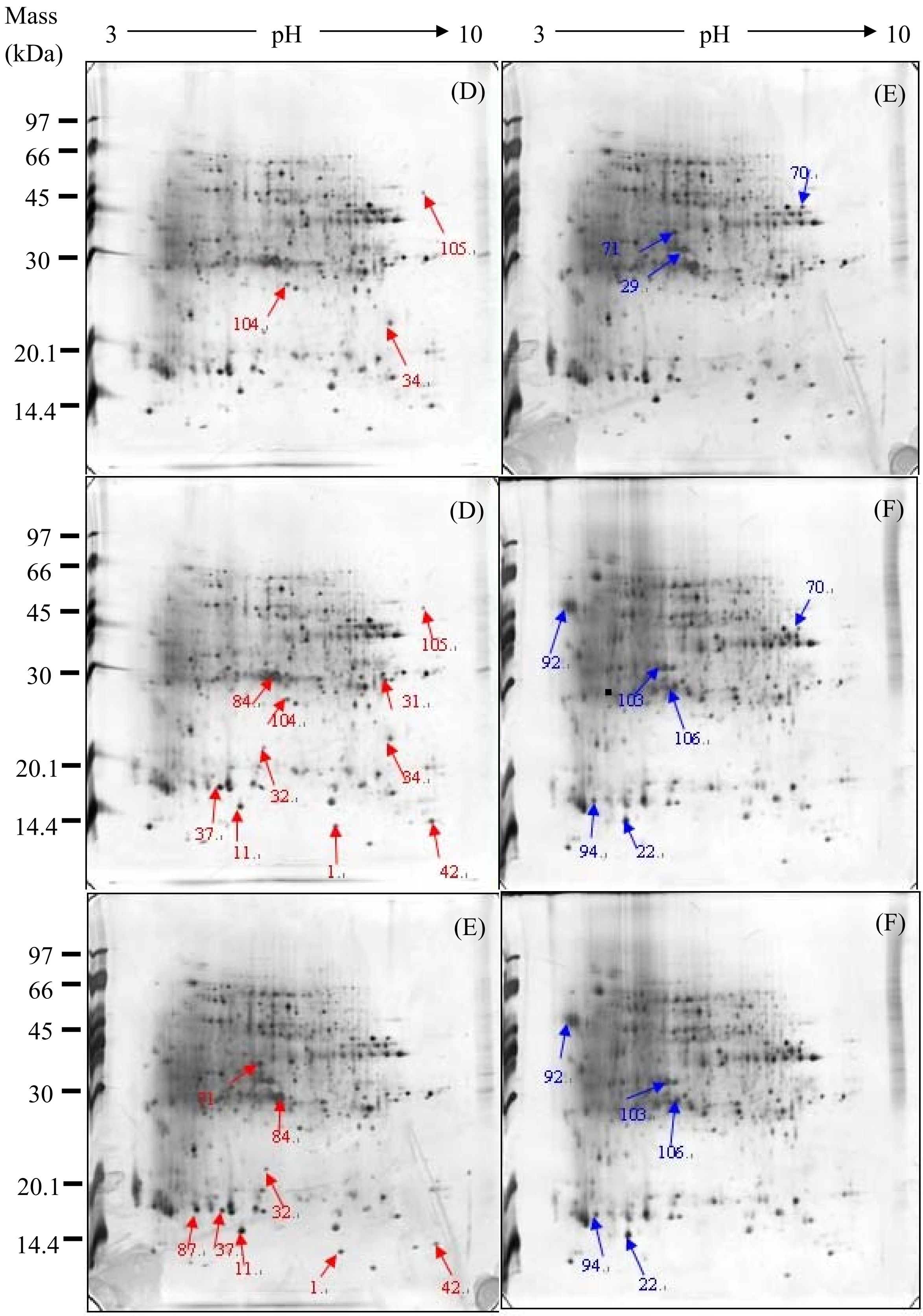
(G) MZ-mice: mice-gall tissues of *Machilus zuihoensis var. zuihoensis*.

(H) MM-mice: mice-gall tissues of *Machilus zuihoensis var. mushaensis.*

(I) MT-mice: mice-gall tissues of *Machilus thunbergii.*

The protein spots labeled are those who differentially expressed by at least ten-fold in comparison with each other.

Note: Experiment was done by Hung-Pin Chen. Data collection and arrangement were done by me.



Mass
(kDa)
3 pH 10
3 pH 10
(D)
(E)
(D)
(F)
(E)
(F)
97
66
45
30
20.1
14.4
105
104
34
70
71
29
84
31
32
37
11
1
42
92
103
106
94
22
87

Figure 16. Protein pattern differences between bell galls of three *Machilus* species.

The bell-galls of three *Machilus* were classified into three groups, (D) and (E), (D) and (F), (E) and (F). The bell-gall protein patterns were compared with each other.**(D)** MZ-bell: bell galls of *Machilus zuihoensis var. zuihoensis*. **(E)** MM-bell: bell galls of *Machilus zuihoensis var. mushaensis.* **(F)** MT-bell: bell galls of *Machilus thunbergii.* Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

Note: Experiment was done by Hung-Pin Chen. Data collection and arrangement were done by me.

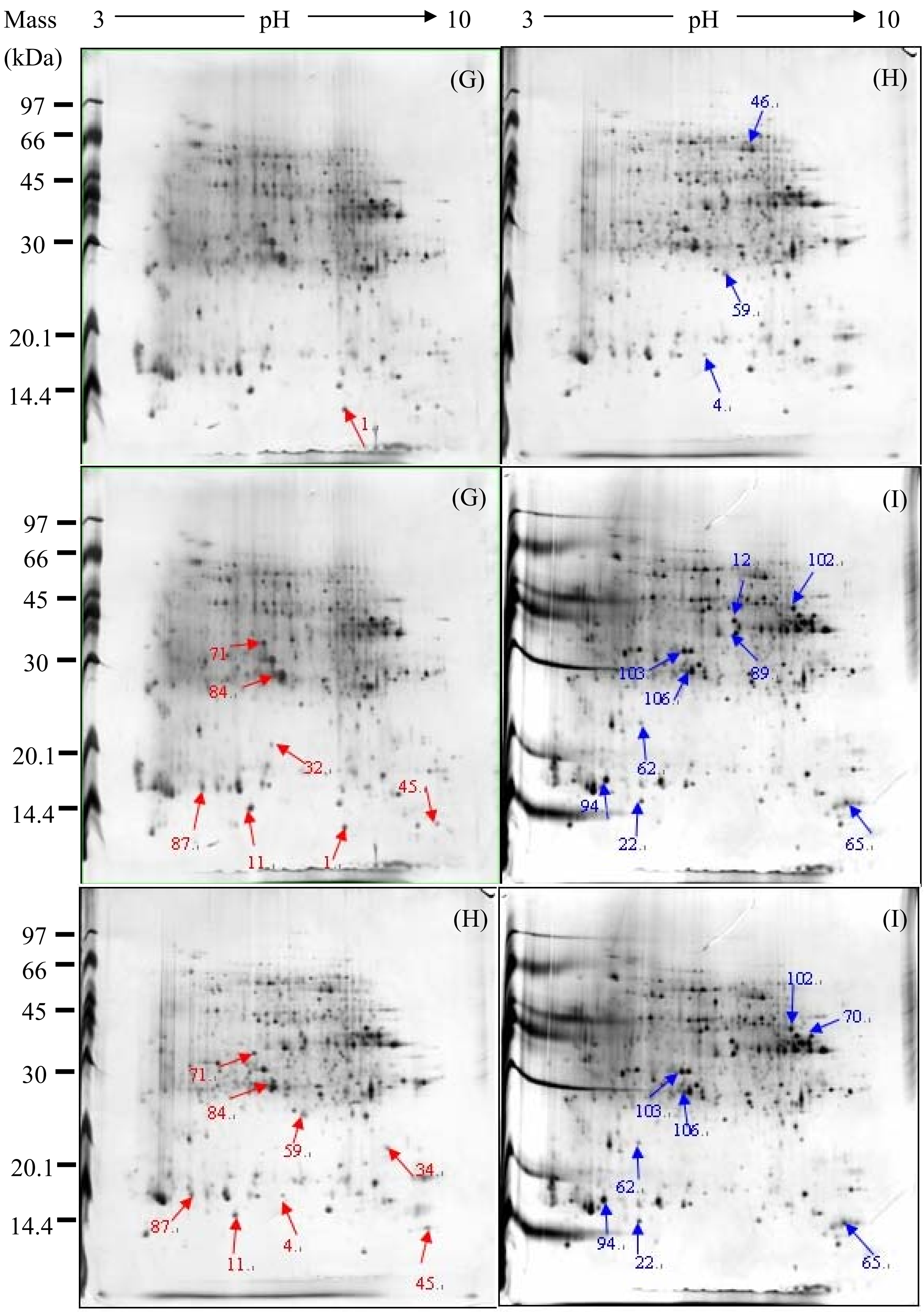

Mass (kDa)
3 pH 10
3 pH 10
97
66
45
30
20.1
14.4
(G)
1
(H)
46
59
4
(G)
71
84
32
45
87
11
1
(I)
12
102
103
89
106
62
94
22
65
(H)
71
84
59
34
87
11
4
45
(I)
102
70
103
106
62
94
22
65

Figure 17. Protein pattern differences between mice galls on three *Machilus* leaves.

The mice galls of three *Machilus* were classified into three groups, (G) and (H), (G) and (I), (H) and (I). The mice gall protein patterns were compared with each other.**(G)** MZ-mice: mice galls of *Machilus zuihoensis var. zuihoensis*. **(H)** MM-mice: mice galls of *Machilus zuihoensis var. mushaensis.* **(I)** MT-mice: mice galls of *Machilus thunbergii.* Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

Note: Experiment was done by Hung-Pin Chen. Data collection and arrangement were done by me.

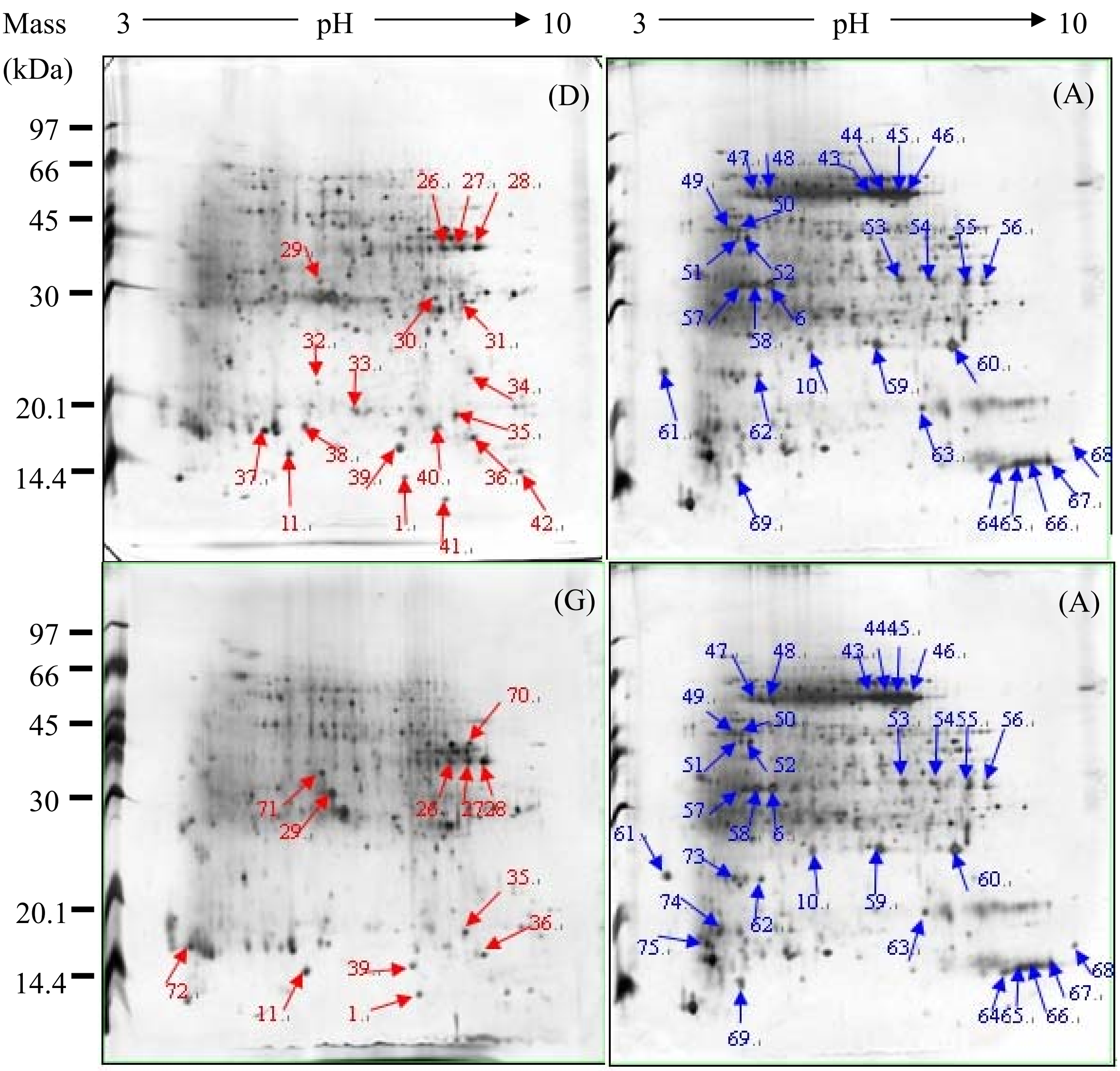


Figure 18. Protein pattern differences between bell-gall-tissues, mice-gall-tissues, and leaf tissues of *Machilus zuihoensis var. zuihoensis*.

Each bell-gall-tissues and mice-gall-tissues were compared with healthy leaf tissues.

(A) MZ-leaf: leaf tissues of *Machilus zuihoensis var. zuihoensis*. (D) MZ-bell : bell galls of *Machilus zuihoensis var. zuihoensis*. (G) MZ-mice: mice galls of *Machilus zuihoensis var. zuihoensis*. Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

Note: Experiment was done by Hung-Pin Chen. Data collection and arrangement were done by me.

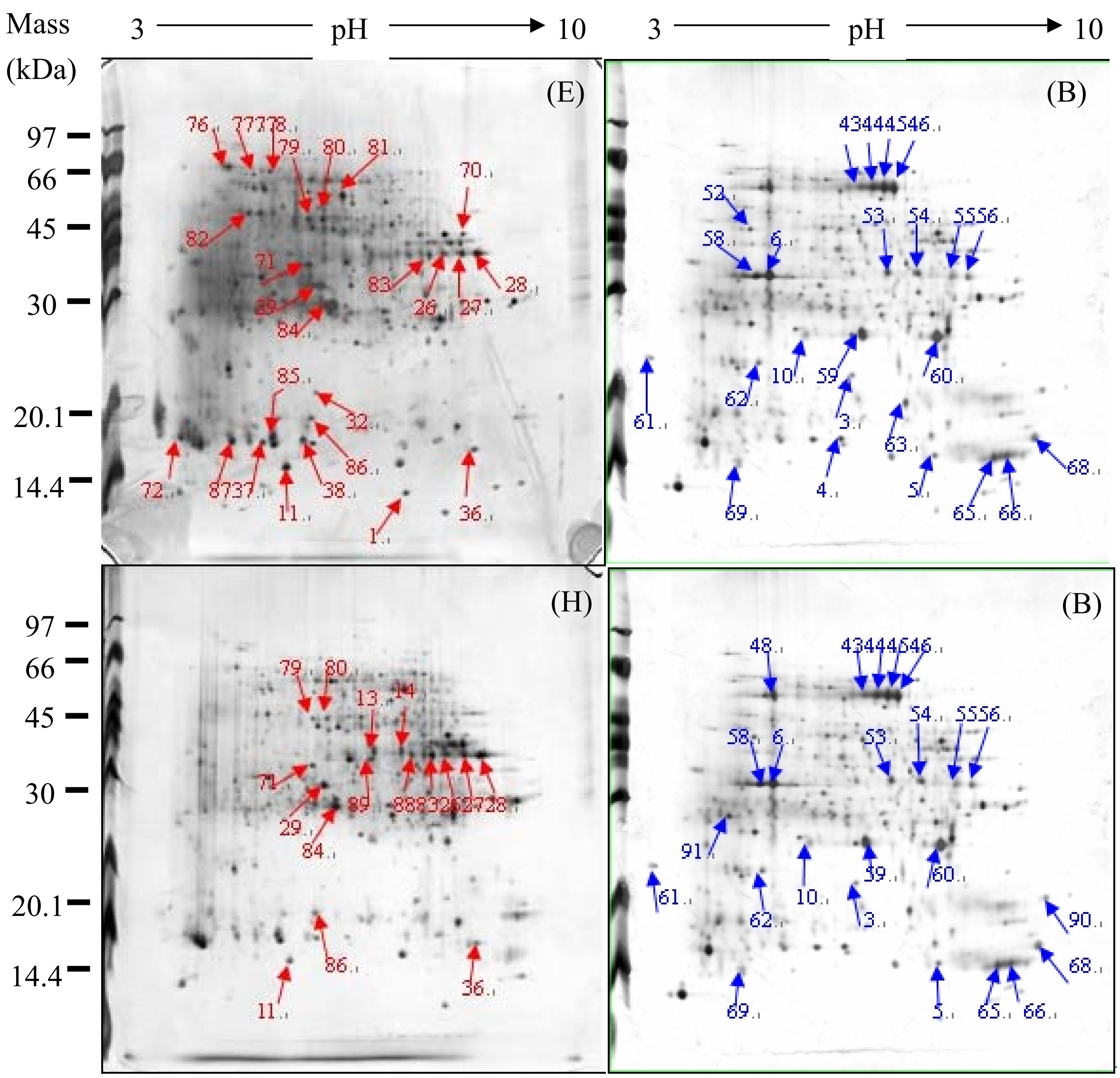


Figure 19. Protein pattern differences between bell-gall-tissues, mice-gall-tissues, and leaf tissues of *Machilus zuihoensis var. mushaensis.*

Each bell-gall-tissues and mice-gall-tissues were compared with healthy leaf tissues.

(B) MM-leaf: leaf tissues of *Machilus zuihoensis var. mushaensis.* **(E)** MM-bell : bell galls of *Machilus zuihoensis var. mushaensis.* **(H)** MM-mice: mice galls of *Machilus zuihoensis var. mushaensis.* Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

Note: Experiment was done by Hung-Pin Chen. Data collection and arrangement were done by me.

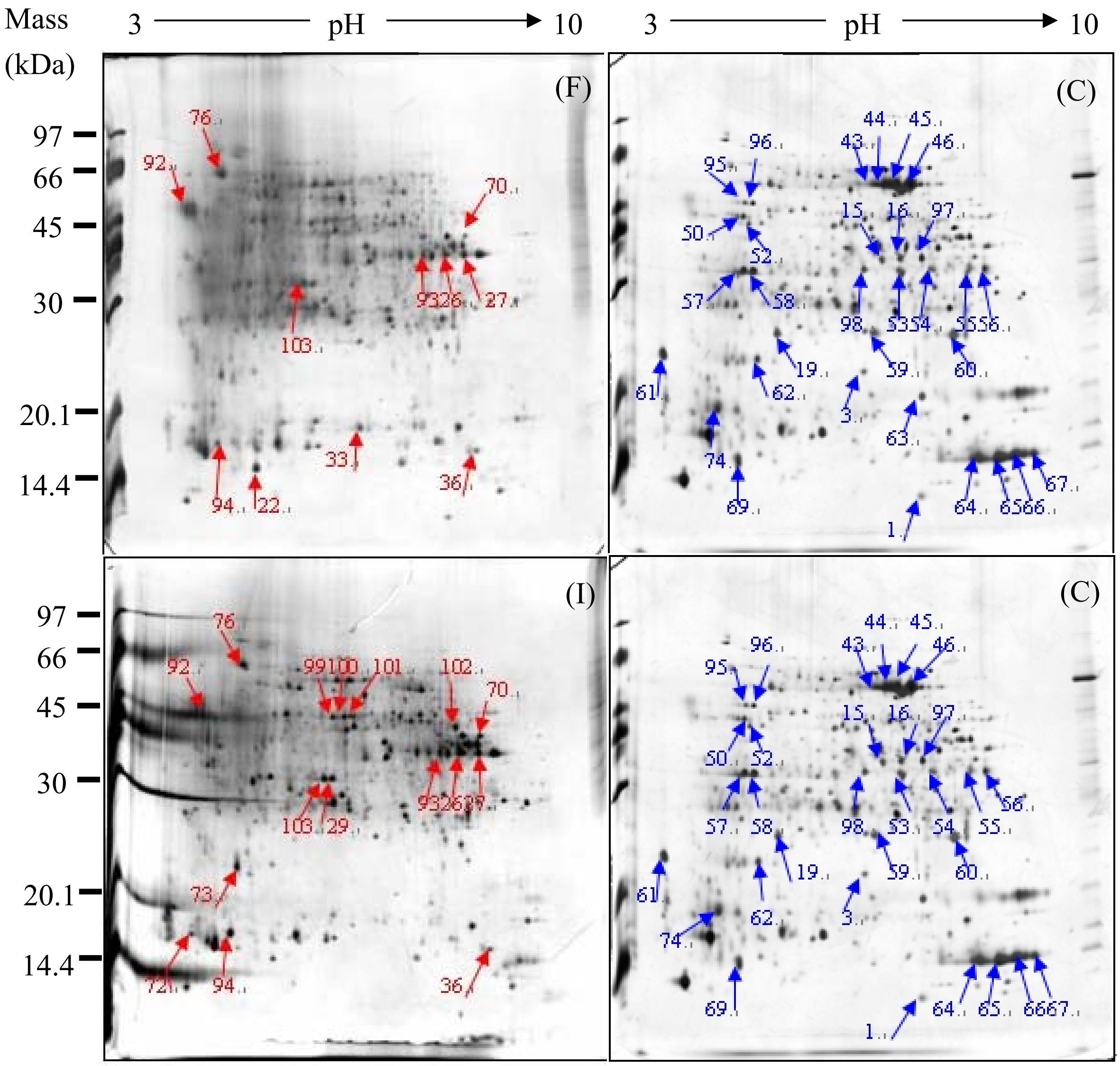
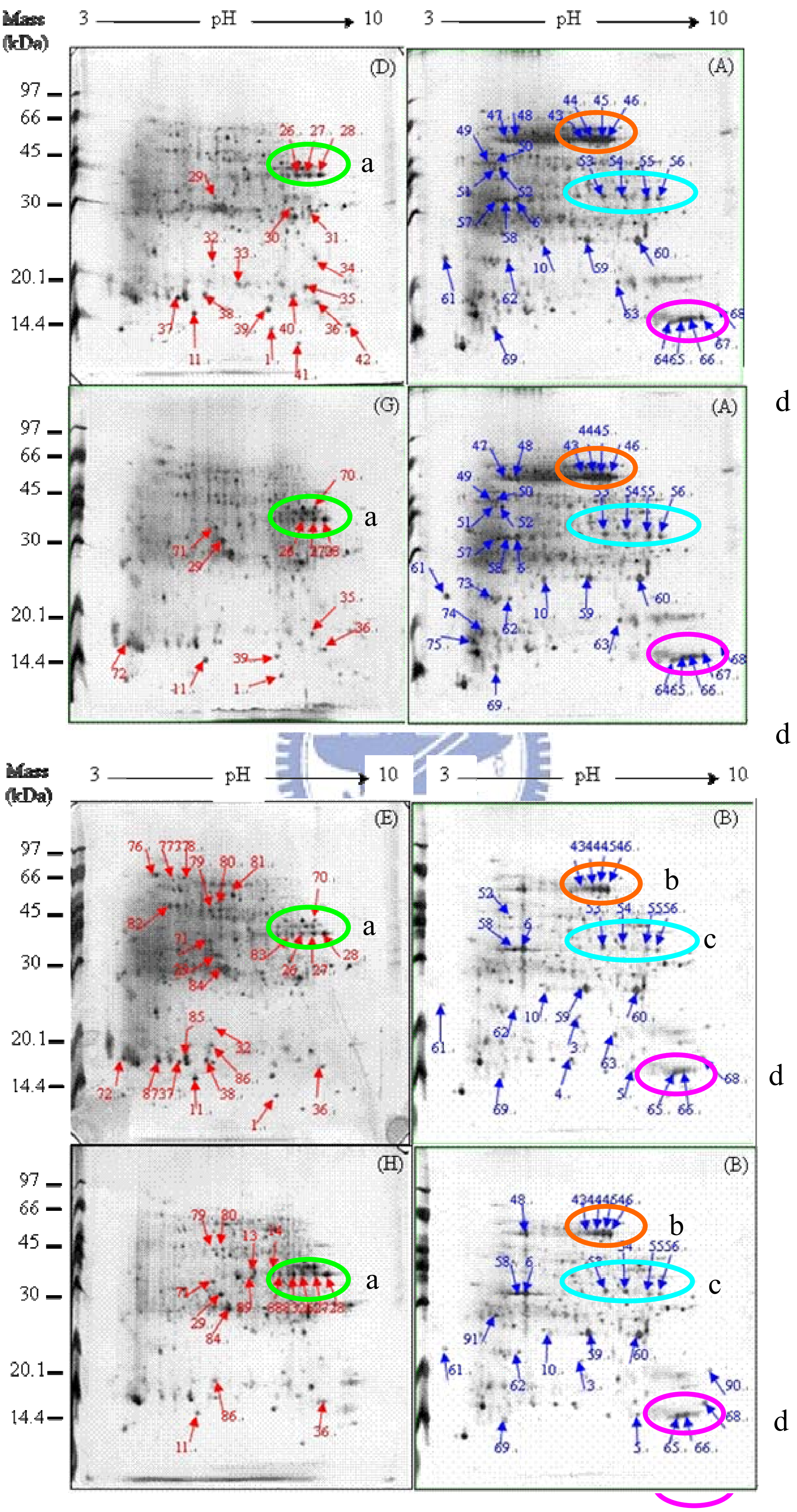


Figure 20. Protein pattern differences between bell-gall-tissues, mice-gall-tissues, and leaf tissues of *Machilus thunbergii.*

Each bell-gall-tissues and mice-gall-tissues were compared with healthy leaf tissues. **(C)** MT-leaf: leaf tissues of *Machilus thunbergii.* **(F)** MT-bell : bell galls of *Machilus thunbergii.* (I) MT-mice: mice galls of *Machilus thunbergii.* Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other.

Note: Experiment was done by Hung-Pin Chen. Data collection and arrangement were done by me.


Mass (kDa)
3
pH
10
97
66
45
30
20.1
14.4
(D)
(A)
(G)
(A)
(E)
(B)
(H)
(B)
a
b
c
d

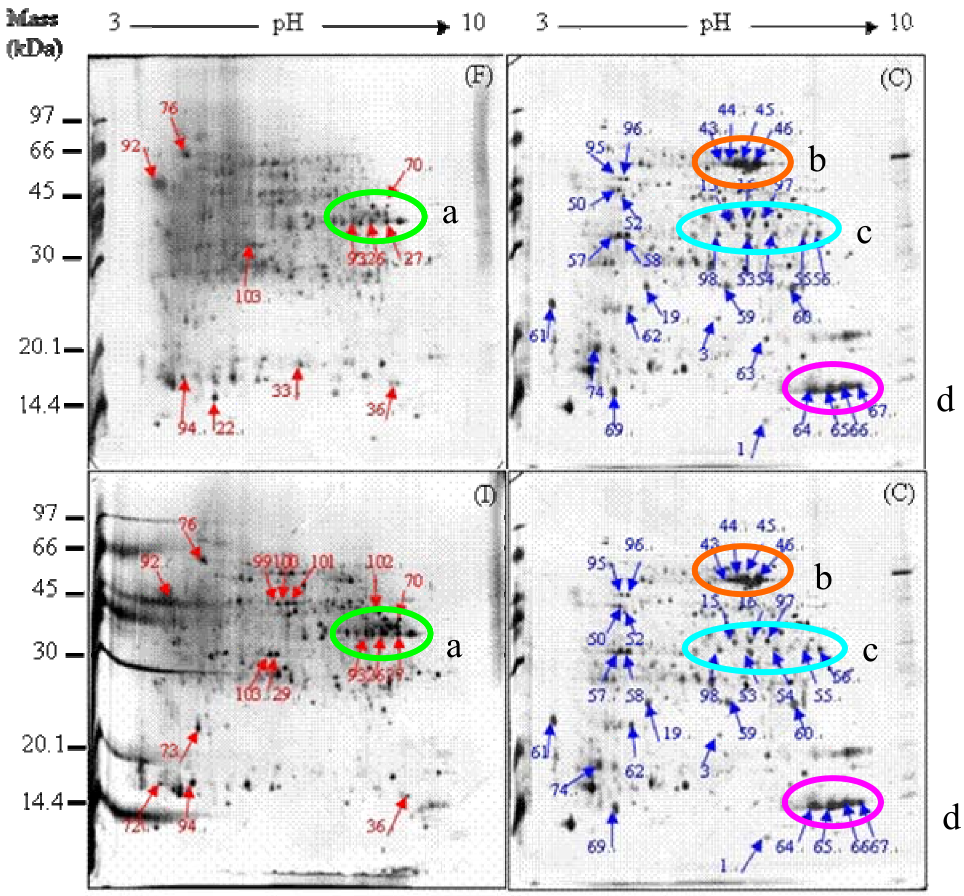


Figure 21. Consensus proteins which appeared within each group

We compared the protein pattern differences between two types of gall tissues with healthy leave tissues among 3 *Machilus* species. Although the protein differences are large in each group, if we looked at all 2D images, we can find out that there are several proteins in common within all groups. Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other. According to their positions on 2D gels proteins were classified into 4 groups, group a b c d. Group a is on the left gel, group b, c and d are on the right gel.

(A) MZ-leaf: leaf tissues of *Machilus zuihoensis var. zuihoensis*. **(B)** MM-leaf: leaf tissues of *Machilus zuihoensis var. mushaensis.* **(C)** MT-leaf: leaf tissues of *Machilus thunbergii.* **(D)** MZ-bell: bell galls of *Machilus zuihoensis var. zuihoensis*. **(E)** MM-bell: bell galls of

Machilus zuihoensis var. mushaensis. **(F)** MT-bell: bell galls of *Machilus thunbergii*.**(G)** MZ-mice: mice galls of *Machilus zuihoensis var. zuihoensis*. **(H)** MM-mice: mice galls of *Machilus zuihoensis var. mushaensis.* Labeled protein spots of the gels are those differentially expressed by at least ten-fold in comparison with each other. **(I)** MT-mice: mice galls of *Machilus thunbergii.*

Note: Experiment was done by Hung-Pin Chen. Data collection and arrangement were done by me.

Table 1. The collection and classification table of gall midges (Cecidomyiidae) on *Machilus* in Taiwan.

Gall type shape / Host plant	Leaf Gall						Stem Gall		
	 1. mice	 2. bell	 3. bird	 4. club	 5. bulb	 6. blister	 7. bud	 8. spindle	 9. bullet
1. MZ	11.# Fu-Shan	12.# Fu-Shan			*15.# Fu-Shan	16	17.# Hua-Hsia	18.# Fu-Shan	19.# Hua-Hsia
2. MM	21.# Yu-Lao	22.# Fu-Shan			25.# Li-Chia	*26 Tai16 freeway	27	28.# Fu-Shan	29.# Li-Chia
3. MT	31.# Chung-Cheng mountain	32.# Chung-Cheng mountain	*33. Fu-Shan	34.# Chung-Cheng mountain	35	36. Yu-Lao	37.# Chung-Cheng mountain	38. Chung-Cheng mountain	39.# Chung-Cheng mountain
4. MJK	41.# Fen-Chi Lake	42.# Fu-Shan		44. Chung-Cheng mountain	45. Fu-Shan	46	47		49.# Cha-yi 169 freeway
5. MJ	51.# Fu-Shan			54.# Fu-Shan	55.# Fu-Shan	56. Li-Chia		58.# Yu-Lao	59.# Fu-Shan
6. MP				64. Nan-An	*65. Nan-An	66	*67. Nan-An	68.# Nan-An	69.# Nan-An
7. MO				74.# Nan-Jen-Shan		76. Sen-Yung			79.# Sen-Yung
8. MK			83. Fen-Chi Lake				87	88	89

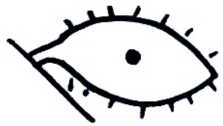
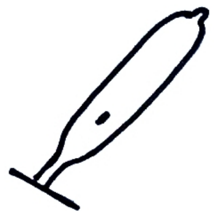
The dots in each sketch represent how many larvae are in one gall. One dot means that there is only one larva in one gall (mice, bell, bird, club, bulb, and bullet). Three dots represent that there are several larvae in one gall (blister, bud, and spindle). Place names are gall-collection sites. We give each host plant and each different type of galls a number, and use those numbers to give our sample a serial number. We name our species-unknown sample according the host plant, gall type and serial number. **1. MZ :** ***Machilus zuihensis Hayata*** **2. MM*: Machilus zuihoensis var. mushaensis*** **3. MT:** ***Machilus thunbergii*** **4. MJK:** ***Machilus japonica kusanoi*** **5. MJ:** ***Machilus japonica*** **6. MP:** ***Machilus philippinense*** **7. MO:** ***Machilus obovatifolia*** **8. MK:** ***Machilus konishii Hayata.*** There are total 40 samples.

* The amount of sample larvae is too small to get PCR results. There are total 35 COI sequence

Samples which were amplified by two sets of primers: COI and 12s.

Columns with only number but no place names represent that the gall morphospecies had been found on this *Machilus*, but we didn't collected this time.

Table 2. *Machilus* gall midges (Cecidomyiidae) used for mitochondrial COI sequences.

Midge Gall Type	Host Plant	Collection Site	Distribution county	Serial Number	sketch
Leaf Galls					
mice	*Machilus zuihensis Hayata*	Fu-Shan	Yilan	11	
mice	*Machilus zuihoensis var. mushaensis*	Yu-Lao	Shinchu	21	
mice	*Machilus thunbergii*	Chung-Cheng mountain	Taipei city	31	
mice	*Machilus japonica kusanoi*	Fen-Chi Lake	Chayi	41	
mice	*Machilus japonica*	Fu-Shan	Yilan	51	
bell	*Machilus zuihensis Hayata*	Fu-Shan	Yilan	12	
bell	*Machilus zuihoensis var. mushaensis*	Fu-Shan	Yilan	22	
bell	*Machilus thunbergii*	Chung-Cheng mountain	Taipei city	32	
bell	*Machilus japonica kusanoi*	Fu-Shan	Yilan	42	
bird	*Machilus konishii Hayata*	Fen-Chi Lake	Chayi	83	
club	*Machilus thunbergii*	Chung-Cheng mountain	Taipei city	34	
club	*Machilus japonica kusanoi*	Chung-Cheng mountain	Taipei city	44	
club	*Machilus japonica*	Fu-Shan	Yilan	54	
club	*Machilus philippinense*	Nan-An	Hualian	64	
club	*Machilus obovatifolia*	Nan-Jen-Shan	Pintung	74	

bulb	*Machilus zuihoensis var. mushaensis*	Li-chia	Taitung	25
bulb	*Machilus japonica kusanoi*	Fu-Shan	Yilan	45
bulb	*Machilus japonica*	Fu-Shan	Yilan	55

blister	*Machilus thunbergii*	Yu-Lao	Shinchu	36
blister	*Machilus japonica*	Li-Chia	Taitung	56
blister	*Machilus obovatifolia*	Sen-Yung	Taitung	76

Stem Galls

bud	*Machilus zuihensis Hayata*	Hua-Hsia	Taipei	17
bud	*Machilus thunbergii*	Chung-Cheng mountain	Taipei city	37
spindle	*Machilus zuihensis Hayata*	Fu-Shan	Yilan	18
spindle	*Machilus zuihoensis var. mushaensis*	Fu-Shan	Yilan	28
spindle	*Machilus thunbergii*	Chung-Cheng mountain	Taipei city	38
spindle	*Machilus japonica*	Yu-Lao	Shinchu	58
spindle	*Machilus philippinense*	Nan-An	Hualian	68
bullet	*Machilus zuihensis Hayata*	Hua-Hsia	Taipei	19

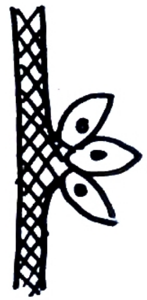
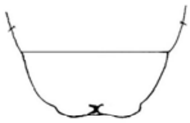
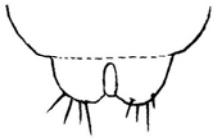
bullet	*Machilus zuihoensis var. mushaensis*	Li-Chia	Taitung	29	
bullet	*Machilus thunbergii*	Chung-Cheng mountain	Taipei city	39	
bullet	*Machilus japonica kusanoi*	Cha-Yi 169 freeway	Chayi	49	
bullet	*Machilus japonica*	Fu-Shan	Yilan	59	
bullet	*Machilus philippinense*	Nan-An	Hualian	69	
bullet	*Machilus obovatifolia*	Sen-Yung	Taitung	79	

Table 2. *Machilus* gall midges (Cecidomyiidae) used for mitochondrial COI sequences.

The dots in each sketch represent how many larvae are in one gall. One dot means there is only one larva in one gall. Three dots represent that there are more than one larva in one gall. We give each host plant and each different type of galls a number, and use those numbers to give our sample a serial number. We name our species-unknown sample according the host plant, gall type and serial number. The number of host plant and gall type are as table 1. The geographic position of collection site are as in Fig. 1.

Table 3. The four major morphological types of Cecidomyiidae larvae in last stage.

	Type I	Type II	Type III	Type IV
Anus				
Spatula				

The for mahor morphologica. Types of Cecidomyiidae larvae in last stage. They contains four different types of Spatulas and Anuses.

Table source: Dr. Tung in TFRI.

Table 4. Characteristics of cecidomyiidae larvae

Gall Type	Segmentation	Spatula (type)	Anus (type)	Spiracle	Papillae (neck seg.)	Papillae (thoracic seg.)
mice	1+1+3+9	I	I	1+8	0	2+4+7
bell	1+1+3+9	I	I	1+8	2	6+8+2
club	1+1+3+9	I	I	1+8	0	2+6+8
bird	1+1+3+9	I	I	1+8	0	2+6+8
bulb	1+1+3+9	I	I	1+8	0	2+6+8
bullet	1+1+3+9	I	I	1+8	2	6+2+2
blister	1+1+3+9	III	III	1+8	0	2+4+6
Ball_bud	1+1+3+9	II	II	1+8	0	4+4+2
Long_bud	1+1+3+9	III	III	1+8	0	4+6+2
spindle	1+1+3+9	IV	IV	1+8	0	0+0+10

The Roman numerals in Spatula and Anus columns are larvae types classified in Table 3.

Table source: Dr. Tung in TFRI.

Table 5. Taxonomic table of *Machilus* gall-midge (cecidomyiidae) by COI gene.

Tribe	A.S.D(%)	Genus	A.S.D.(%)	Species	A.S.D.(%)	Collection site
T1. mz-bud	25	G1		S1. mz-bud 17		Hua-Hsia
T2. mt-bud	25	G2		S2. mt-bud 37		Chung-Cheng mountain
T3. mt-blister	23	G3		S3. mt-blister 36		Yu-Lao
T4. mj-blister	23	G4		S4. mj-blister 56		Li-Chia
T5. mo-blister	23	G5		S5. mo-blister 76		Sen-Yung
T6 spindle	20	G6	11	S6. mt-spindle 38	0.25	Chung-Cheng mountain
				S6. mp-spindle 68		Nan-An
				S7. mm-spindle 28	0.51	Fu-Shan
				S7. mz-spindle 18		Fu-Shan
				S7. mj-spindle 58		Yu-Lao
T7. Compounded	20	G7-bulb	9.73	S8. mjk-bulb 45	2.83	Fu-Shan
				S8. mj-bulb 55		Fu-Shan
				S9. mm-bulb 25		Li-Chia

G8-bullet-1	12	S10.mz-bullet 19	0.26	Hua-Hsia
		S10.mm-bullet 29		Li-Chia
		S11.mjk-bullet 49	0.6	Cha-Yi 169 freeway
		S11.mp-bullet 69		Nan-An
		S11.mo-bullet 79		Sen-Yung
G9-bullet-2	12	S12. mt-bullet 39		Chung-Cheng mountain
		S13. mj-bullet 59		Fu-Shan
G10-mice		S14. mz-mice 11	0	Fu-Shan
		S14. mm-mice 21		Yu-Lao
		S14. mjk-mice 31		Chung-Cheng mountain
		S14. mj-mice 41		Fen-Chi Lake
		S14. mt-mice 51		Fu-Shan
G11-bell club bird		S15. mz-bell 12	2	Fu-Shan
		S15. mm-bell 22		Fu-Shan
		S15. mt-bell 32		Chung-Cheng mountain
		S15. mjk-bell 42		Fu-Shan
		S15. mt-club 34		Chung-Cheng mountain
		S15. mjk-club 44		Chung-Cheng mountain
		S15. mj-club 54		Fu-Shan
		S15. mp-club 64		Nan-An

		S15. mo-club 74	Nan-Jen-Shan
		S15. Mk-bird 83	Fen-Chi lake

Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. According to the phylogenic tress, species on top of the table are early differentiated species, and species at the button are later differentiated species.

1.MZ : *Machilus zuihensis Hayata.* **2.MM:** *Machilus zuihoensis. var. mushaensis.* **3.MT**: *Machilus thunbergii.* **4.MJK:** *Machilus japonica kusanoi.* **5.MJ:** *Machilus japonica.* **6.MP:** *Machilus philippinense.* **7.MO:** *Machilus obovatifolia.* ***8.*MK**: *Machilus konishii Hayata.* Letters after dashed line are gall types.1.mice. 2.bell. 3.bird. 4.club. 5.bulb. 6.blister. 7.bud. 8.spindle. 9.bullet. A.S.D.: Average sequence divergence per 100 bp compared with same level. A.S.D. With in species is about 0.25% (same species). A.S.D. between species is about 6-13% (same genus). A.S.D. between genera is about 11-17% (same tribe). A.S.D. between tribes is above 17% (same family).

Table 6. Taxonomic table of *Machilus* gall-midge (cecidomyiidae) by 12S gene.

Tribe	A.S.D(%)	Genus	A.S.D.(%)	Species	A.S.D.(%)	Collection site
T1. bud		G1-bud		S1. mt-bud 37		Chung-Cheng mountain
				S1. mz-bud17		Hua-Hsia
T2. spindle		G2-spindle		S2. mz-spindle18		Fu-Shan
				S2. mm-spindle28		Fu-Shan
				S3. mj-spindle58		Yu-Lao
				S4. mp-spindle68		Nan-An
T3. bulb		G3-bulb-1	5.76	S5. mm-bulb25		Li-Chia
				S5. mj-bulb55		Fu-Shan
		G4-bulb-2		S6. mz-bulb15		Fu-Shan
T4. bullet		G5-bullet-1		S7. mt-bullet39		Chung-Cheng mountain
				S7. mj-bullet59		Fu-Shan
		G6-bullet-2		S8. mp-bullet69		Nan-An
				S8. mjk-bullet 49		Cha-Yi 169 freeway
				S8. mo-bullet79		Sen-Yung

	G7-bullet-3	S9. mz-bullet19	Hua-Hsia
		S9. mm-bullet29	Li-Chia
T5. mice	G8-mice-1	S10. mjk-mice41	Fen-Chi Lake
		S10. mt-mice31	Chung-Cheng mountain
	G9-mice-2	S11. mj-mice51	Fu-Shan
		S11. mz-mice11	Fu-Shan
	G10-mice-3	S12. mm-mice21	Yu-Lao
T6 Compounded	G11-bell	S13. mt-bell32	Chung-Cheng mountain
		S13. mm-bell22	Fu-Shan
		S13. mjk-bell42	Fu-Shan
	G12-club bulb	S14 mo-club74	Nan-Jen-Shan
		S14 mt-club34	Chung-Cheng mountain
		S15 mj-club54	Fu-Shan
		S16 mz-bell12	Fu-Shan

Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. According to the phylogenic tress, species on top of the table are early differentiated species, and species at the button are later differentiated.

1.MZ : *Machilus zuihensis Hayata.* **2.MM*:*** *Machilus zuihoensis. var. mushaensis.* **3.MT**: *Machilus thunbergii.* **4.MJK:** *Machilus japonica kusanoi.* **5.MJ:** *Machilus japonica.* **6.MP:** *Machilus philippinense.* **7.MO:** *Machilus obovatifolia.* ***8.*MK**: *Machilus konishii Hayata.* Letters after dashed line are gall types.1.mice. 2.bell. 3.bird. 4.club. 5.bulb. 6.blister. 7.bud. 8.spindle. 9.bullet. A.S.D.: Average sequence divergence per 100 bp compared with same level. A.S.D. With in species is about 0.25% (same species). A.S.D. between species is about 6-13% (same genus). A.S.D. between genera is about 11-17% (same tribe). A.S.D. between tribes is above 17% (same family).

Table 7. Protein pattern differences between bell and mice galls on single *Machilus* species

Plant-gall type	Protein increased	Protein increased	Plant-gall type
MZ-bell	34、104、105	29、70、71	**MZ-mice**
MM-bell	5、70、37	34、46	**MM-mice**
MT-bell	None	65、94、102	**MT-mice**

MZ: *Machilus zuihoensis var. zuihoensis*. **MM**: *Machilus zuihoensis var. mushaensis.* **MT:** *Machilus thunbergii.* Numbers are protein spots labeled in **Fig 2.**

Table 8. Protein pattern differences between bell galls on three *Machilus* leaves

Plant-gall type	Protein increased	Protein increased	Plant-gall type
M.Z.-bell	34、104、105 Total: 3 proteins	29、70、71 Total: 3 proteins	**M.M-bell**
M.Z.-bell	**1**、**11**、31、32、34、37、42、84、104、105 Total:10 proteins	**22**、70、92、94、103、106 Total:6 proteins	**M.T-bell**
M.M-bell	1、**11**、32、37、42、84、87、37 Total:8 proteins	**22**、92、94、103、106 Total:5 proteins	**M.T-bell**

MZ: *Machilus zuihoensis var. zuihoensis*. **MM**: *Machilus zuihoensis var. mushaensis*. **MT:** *Machilus thunbergii.*

Numbers are protein spots labeled in Fig 2. Boldface numbers are protein differences with plant leaves.

Table 9. Protein pattern differences between mice galls on three *Machilus* leaves

Plant-gall type	Protein increased	Protein increased	Plant-gall type
M.Z-mice	**1**	**4**、46、59	**M.M-mice**
M.Z-mice	**1**、**11**、32、45、71、84、87、 Total:7 proteins	**12**、**22**、62、65、89、94、102、103、106 Total:9 proteins	**M.T-mice**
M.M-mice	**4**、**11**、34、45、59、71、84、87、 Total:8 proteins	**22**、62、65、70、94、102、103、106 Total:8proteins	**M.T-mice**

MZ: *Machilus zuihoensis var. zuihoensis*. **MM**: *Machilus zuihoensis var. mushaensis.* **MT:** *Machilus thunbergii.*

Numbers are protein spots labeled in Fig 2. Boldface numbers are protein differences with plant leaves.

Table 10. Total Protein pattern difference between each sample

	(A) MZ-leaf	(B) MM-leaf	(C) MT-leaf	(D) MZ-bell	(E) MM-bell	(F) MT-bell	(G) MZ-mice	(H)MM-mice	(I) MT-mice
(A)MZ-leaf	-----	2、3、4、5	3、12-25	1、11、26-42	1、11、26-29、32、36-38、70、71、72、76-87	-----	1、11、26-29、35、36、39、70、71、72	-----	-----
(B)MM-leaf	1	-----	1、12-25	-----	1、11、26-29、32、36-38、70-72、76-87	-----	-----	11、13、14、26-29、36、71、79、80、84、86、88、89	-----
(C)MT-leaf	1、6-11	3、6-11	-----	-----	-----	22、26、27、33、36、70、76、92、94、103	-----	-----	26、27、29、36、70、72、73、76、92-94、99-103
(D)MZ-bell	6、43-69	-----	-----	-----	70、71、29	22、70、92、94、103、106	29、70、71	-----	-----
(E)MM-bell	------	3-6、10、	-----	34、104、	-----	22、92、	-----	34、46	-----

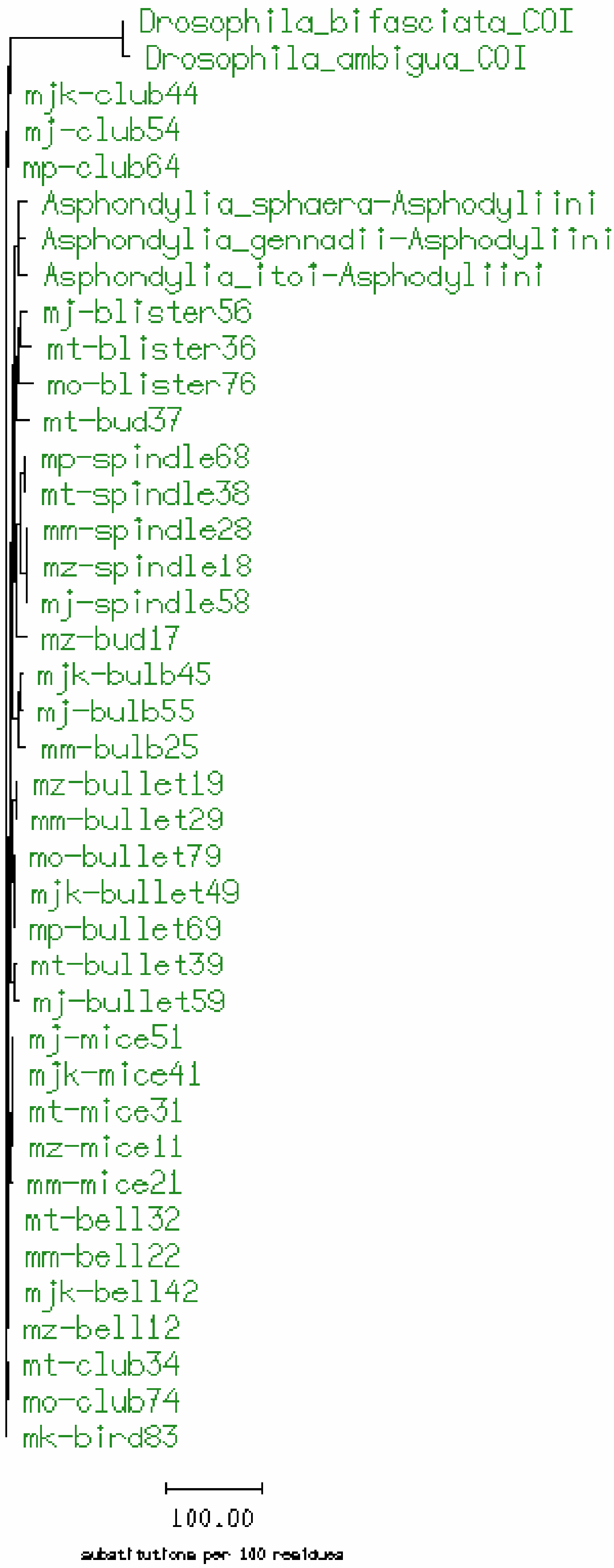
		43-46、52-56、58-63、65、66、68、69		105		94、103、106			
(F)MT-bell	-----	-----	1、3、15、16、19、43-46、50、52-67、69、74、95-98	**1、11**、31、32、34、37、42、84、104、105	1、**11**、32、37、42、84、87、37	-----	-----	-----	65、94、102
(G)MZ-mice	6、10、43-69、73-75	-----	-----	34、104、105	-----	-----	-----	**4**、46、59	**12、22**、62、65、89、94、102、103、106
(H)MM-mice	-----	3、5、6、10、43-48、53-56、58-62、65、66、68、69、90、91	-----	-----	5、37、70	-----	**1**	-----	**22**、62、65、70、94、102、103、106
(I)MT-mice	-----	-----	1、3、15、16、19、43-46、50、52-62、	-----	-----	0	**1、11**、32、45、71、84、87、	**4、11**、34、45、59、71、84、87、	-----

			64-67、69、74、95-98						

(A) MZ-leaf: leaf tissues of *Machilus zuihoensis var. zuihoensis*. (B) MM-leaf: leaf tissues of *Machilus zuihoensis var. mushaensis.* (C) MT-leaf: leaf tissues of *Machilus thunbergii.* (D) MZ-bell: bell-gall tissues of *Machilus zuihoensis var. zuihoensis*. (E) MZ-mice: mice-gall tissues of *Machilus zuihoensis var. zuihoensis*. (F) MM-bell: bell-gall tissues of *Machilus zuihoensis var. mushaensis.* (G) MM-mice: mice-gall tissues of *Machilus zuihoensis var. mushaensis* (H) MT-bell: bell-gall tissues of *Machilus thunbergii* (I) MT-mice: mice-gall tissues of *Machilus thunbergii.*

Each English Letter corresponds to each photograph in Fig. 11-18. Boldface numbers are protein differences with in healthy leaves.

Appendix 1 Further analysis of COI and 12S genes


Drosophila_bifasciata_COI
Drosophila_ambigua_COI
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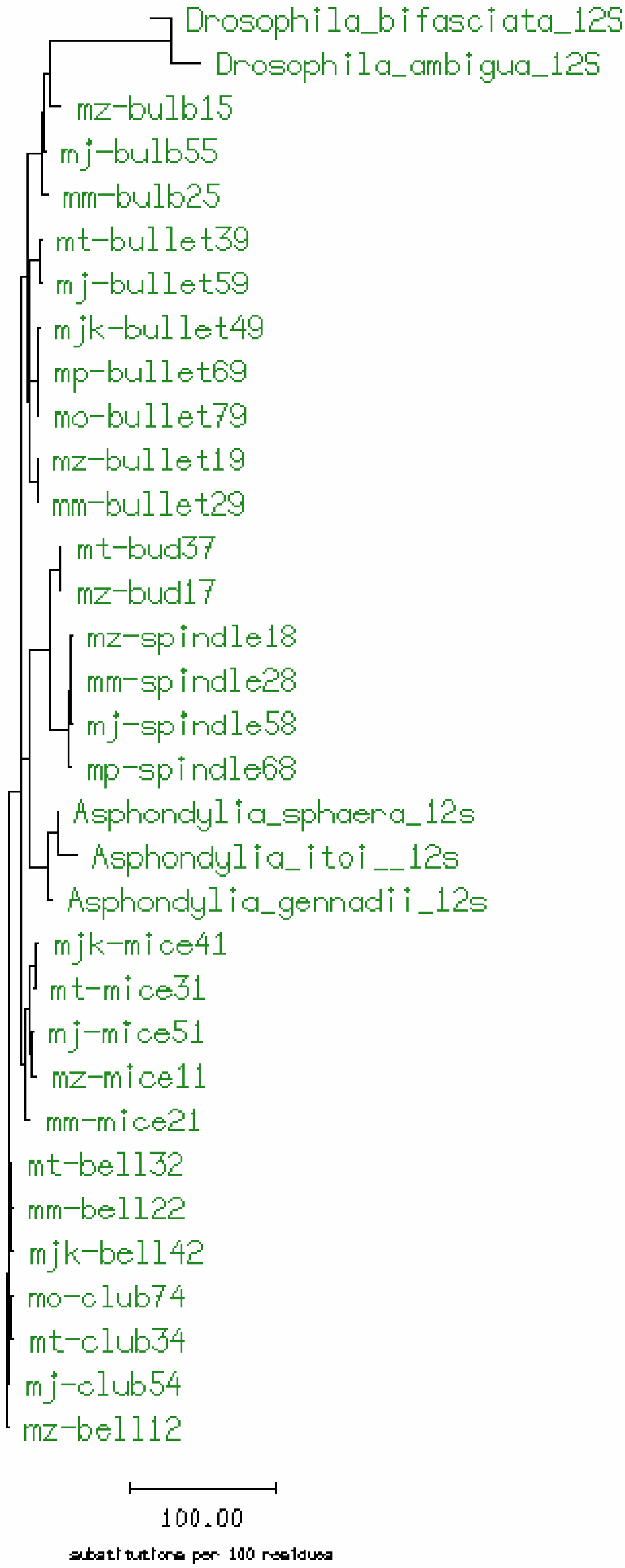
mj-club54
mp-club64
Asphondylia_sphaera-Asphodyliini
Asphondylia_gennadii-Asphodyliini
Asphondylia_itoi-Asphodyliini
mj-blister56
mt-blister36
mo-blister76
mt-bud37
mp-spindle68
mt-spindle38
mm-spindle28
mz-spindle18
mj-spindle58
mz-bud17
mjk-bulb45
mj-bulb55
mm-bulb25
mz-bullet19
mm-bullet29
mo-bullet79
mjk-bullet49
mp-bullet69
mt-bullet39
mj-bullet59
mj-mice51
mjk-mice41
mt-mice31
mz-mice11
mm-mice21
mt-bell32
mm-bell22
mjk-bell42
mz-bell12
mt-club34
mo-club74
mk-bird83
100.00
substitutions per 100 residues

Figure 22. Evolutionary tree of gall midge COI DNA with Neighbor-Joining method and Drosophila outgroups

This neighbor-joining tree for gall-midges of *Machilus* is based on average 396 bp of the mitochondrial COI gene and Kimura's two-parameter distance. There are 5 out-group sequences and 35 sample sequences. Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. **1. MZ :** *Machilus zuihensis Hayata.* **2. MM:** *Machilus zuihoensis. var. mushaensis.* **3. MT**: *Machilus thunbergii.* **4. MJK:** *Machilus japonica kusanoi.* **5. MJ:** *Machilus japonica.* **6. MP:** *Machilus philippinense.* **7. MO:** *Machilus obovatifolia.* ***8.*** **MK**: *Machilus konishii Hayata.* Letters after dashed line are gall types.1.mice. 2. bell. 3. bird. 4. club. 5. bulb. 6. blister. 7. bud. 8. spindle. 9. bullet. The dots in each sketch represent how many larvae are in one gall. One dot means there is only one larva in one gall. Three dots represent that there are more than one larva in one gall. Numbers beside sketches are larva types in table 4.


Drosophila_bifasciata_12S
Drosophila_ambigua_12S
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mj-bulb55
mm-bulb25
mt-bullet39
mj-bullet59
mjk-bullet49
mp-bullet69
mo-bullet79
mz-bullet19
mm-bullet29
mt-bud37
mz-bud17
mz-spindle18
mm-spindle28
mj-spindle58
mp-spindle68
Asphondylia_sphaera_12s
Asphondylia_itoi__12s
Asphondylia_gennadii_12s
mjk-mice41
mt-mice31
mj-mice51
mz-mice11
mm-mice21
mt-bell32
mm-bell22
mjk-bell42
mo-club74
mt-club34
mj-club54
mz-bell12
100.00
substitutions per 100 residues

Figure 23. Evolutionary tree of gall midge 12S DNA with Neighbor-Joining method and Drosophila outgroups

This neighbor-joining tree for gall-midges of *Machilus* is based on average 430 bp of the mitochondrial 12s gene and Kimura's two-parameter distance. There are 5 out-group sequences and 28 sample sequences. Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. **1.MZ :** *Machilus zuihensis Hayata.* **2.MM:** *Machilus zuihoensis. var. mushaensis.* **3.MT**: *Machilus thunbergii.* **4.MJK:** *Machilus japonica kusanoi.* **5.MJ:** *Machilus japonica.* **6.MP:** *Machilus philippinense.* **7.MO:** *Machilus obovatifolia.* ***8.*MK**: *Machilus konishii Hayata.* Letters after dash line are gall types.1.mice. 2.bell. 3.bird. 4.club. 5.bulb. 6.blister. 7.bud. 8.spindle. 9.bullet. The dots in each sketch represent how many larvae are in one gall. One dot means there is only one larva in one gall. Three dots represent that there are more than one larva in one gall. Numbers beside sketches are larva types in table 5.

Extended majority rule consensus tree

CONSENSUS TREE:
the numbers on the branches indicate the number
of times the partition of the species into the two sets
which are separated by that branch occurred
among the trees, out of 100.00 trees

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                                                                                                                  +------mp-bu69
                                                                                                           +-71.0-|
                                                                                                    +-83.0-|      +------mjk-bu49
                                                                                                    |      |
                                                                 +-----------------------------67.0-|      +-------------mo-bu79
                                                                 |                                  |
                                                                 |                                  |             +------mz-bu19
                                                                 |                                  +--------90.0-|
                                                                 |                                                +------mm-bu29
                                                                 |
                                                                 |                                                +------mj-club54
                                                                 |                                         +-57.0-|
                                                                 |                                  +-90.0-|      +------mp-club64
                                                                 |                                  |      |
                                                                 |                           +-70.0-|      +-------------mjk-club44
                                                                 |                           |      |
                                                                 |                           |      |             +------mo-club74
                                                                 |                    +-53.0-|      +--------92.0-|
                                                                 |                    |      |                    +------mt-club34
                                                          +-60.0-|                    |      |
                                                          |      |                    |      |                    +------mz-bell12
                                                          |      |             +-99.0-|      +---------------51.0-|
                                                          |      |             |      |                           +------mk-bird83
                                                          |      |             |      |
                                                          |      |             |      |                           +------mjk-bell42
                                                          |      |             |      |                    +-57.0-|
                                                          |      |             |      +---------------97.0-|      +------mt-bell32
                                                          |      |             |                           |
                                                          |      |      +-87.0-|                           +-------------mm-bell22
                                                          |      |      |      |
                                                          |      |      |      |                                  +------mj-mice51
                                                          |      |      |      |                           +-58.0-|
                                                   +-53.0-|      |      |      |                    +-65.0-|      +------mjk-mice41
                                                   |      |      |      |      |                    |      |
                                                   |      |      +-60.0-|      |             +100.0-|      +-------------mt-mice31
                                                   |      |             |      |             |      |
                                                   |      |             |      |             |      |
                                                   |      |             |      +-------100.0-|      +--------------------mz-mice11
                                                   |      |             |                    |
                                                   |      |             |                    +---------------------------mm-mice21
                                                   |      |             |
                                                   |      |             |                                         +------mt-bu39
                                            +-33.0-|      |             +------------------------------------99.0-|
                                            |      |      |                                                       +------mj-bu59
                                            |      |      |
                                            |      |      |                                                       +------mj-bulb55
                                            |      |      |                                                +-96.0-|
                                            |      |      +------------------------------------------100.0-|      +------mjk-bulb45
                                            |      |                                                       |
                                     +-29.0-|      |                                                       +-------------mm-bulb25
                                     |      |      |
                                     |      |      |                                                              +------D bifascia
                                     |      |      +--------------------------------------------------------100.0-|
                                     |      |                                                                     +------D ambigua
                                     |      |
                              +-26.0-|      |                                                                     +------A gennadii
                              |      |      |                                                              +-58.0-|
                              |      |      +---------------------------------------------------------98.0-|      +------A sphaera
                              |      |                                                                     |
                       +-35.0-|      |                                                                     +-------------A itoi
                       |      |      |
                       |      |      +-----------------------------------------------------------------------------------mz-bud17
                       |      |
                       |      |                                                                            +-------------mt-bud37
                +-92.0-|      +-----------------------------------------------------------------------21.0-|
                |      |                                                                                   |      +------mj-bl56
                |      |                                                                                   +-44.0-|
         +100.0-|      |                                                                                          +------mt-bl36
         |      |      |
         |      |      +-------------------------------------------------------------------------------------------------mo-bl76
         |      |
         |      |                                                                                                 +------mt-sp38
  +------|      +--------------------------------------------------------------------------------------------99.0-|
  |      |                                                                                                        +------mp-sp68
  |      |
  |      |                                                                                                        +------mm-sp28
  |      +---------------------------------------------------------------------------------------------------99.0-|
  |                                                                                                               +------mz-sp18
  |
  +----------------------------------------------------------------------------------------------------------------------mj-sp58
```

This is an unrooted tree

Figure 24. Bootstrap analysis of COI gene neighbor-joining tree by using Phylip 3.6

This neighbor-joining tree for gall-midges of *Machilus* is based on average 396 bp of the mitochondrial COI gene and Kimura's two-parameter distance. There are 5 out-group sequences and 35 sample sequences. Bootstrap andalysis was done by using phylip 3.6.

Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. **1. MZ :** *Machilus zuihensis Hayata.* **2. MM:** *Machilus zuihoensis. var. mushaensis.* **3. MT**: *Machilus thunbergii.* **4. MJK:** *Machilus japonica kusanoi.* **5. MJ:** *Machilus japonica.* **6. MP:** *Machilus philippinense.* **7. MO:** *Machilus obovatifolia.* ***8.*** **MK**: *Machilus konishii Hayata.* Letters after dashed line are gall types.1.mice. 2. bell. 3. bird. 4. club. 5. bulb. 6. blister. 7. bud. 8. spindle. 9. bullet. The dots in each sketch represent how many larvae are in one gall. One dot means there is only one larva in one gall. Three dots represent that there are more than one larva in one gall. Numbers beside sketches are larva types in table 4.

Since the maxium number of sample name is 10 charecters in phylip program, there are some shortence of some sample name. "Bullet" is shortened to "bu". "Spindle" is shortened to "sp". The name of 5 outgroups are also shortened.

Consensus tree program, version 3.67

Species in order:

1. mj-sp58
2. mp-sp68
3. mt-sp38
4. mj-bl56
5. mt-bl36
6. mo-bl76
7. mt-bud37
8. mz-bud17
9. D bifascia
10. D ambigua

11. A sphaera
12. A gennadii
13. A itoi
14. mm-bulb25
15. mj-bulb55
16. mjk-bulb45
17. mm-bu29
18. mz-bu19
19. mjk-bu49
20. mp-bu69

21. mo-bu79
22. mt-bu39
23. mj-bu59
24. mt-mice31
25. mjk-mice41
26. mj-mice51
27. mz-mice11
28. mm-mice21
29. mjk-bell42
30. mt-bell32

31. mm-bell22

32. mz-bell12
33. mp-club64
34. mjk-club44
35. mj-club54
36. mk-bird83
37. mo-club74
38. mt-club34
39. mm-sp28
40. mz-sp18

Sets included in the consensus tree

Set (species in order)   How many times out of   100.00

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Sets NOT included in consensus tree:

Set (species in order)   How many times out of   100.00

....**..... ....   37.00
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Extended majority rule consensus tree

CONSENSUS TREE:
the numbers on the branches indicate the number
of times the partition of the species into the two sets
which are separated by that branch occurred
among the trees, out of 100.00 trees

```
                                                                                    +------mt-bu39
                                                                      +-------100.0-|
                                                                      |             +------mj-bu59
                                                               +-87.0-|
                                                               |      |      +-------------mo-bu79
                                                               |      +100.0-|
                                                               |             |      +------mp-bu69
                                                 +--------86.0-|             +-78.0-|
                                                 |             |                    +------mjk-bu49
                                                 |             |
                                                 |             |                    +------mz-bu19
                                                 |             +--------------100.0-|
                                                 |                                  +------mm-bu29
                                                 |
                                          +-69.0-|                                  +------mz-mice11
                                          |      |                           +-99.0-|
                                          |      |      +--------------100.0-|      +------mj-mice51
                                          |      |      |                    |
                                          |      |      |                    +-------------mm-mice21
                                          |      |      |
                                          |      |      |                           +------mt-bell32
                                          |      |      |                    +-67.0-|
                                          |      +-78.0-|             +100.0-|      +------mm-bell22
                                          |             |             |      |
                                          |             |             |      +-------------mjk-bell42
                                          |             |      +-52.0-|
                                   +-87.0-|             |      |      |             +------mj-club54
                                   |      |             |      |      |      +-62.0-|
                                   |      |             |      |      +-44.0-|      +------mp-club64
                                   |      |             +100.0-|             |
                                   |      |                    |             +-------------mz-bell12
                                   |      |                    |
                                   |      |                    |                    +------mo-club74
                                   |      |                    +---------------44.0-|
                                   |      |                                         +------mt-club34
                                   |      |
                                   |      |                                         +------mt-mice31
                            +-96.0-|      |                           +-------100.0-|
                            |      |      |                           |             +------mjk-mice41
                            |      |      +----------------------71.0-|
                            |      |                                  |             +------mj-bulb55
                            |      |                                  |      +-49.0-|
                            |      |                                  +-82.0-|      +------mz-bulb15
                            |      |                                         |
                            |      |                                         +-------------mm-bulb25
                            |      |
                            |      |                           +---------------------------A.sph-12S
                     +100.0-|      |                           |
                     |      |      |                           |                    +------D.amb-12S
                     |      |      +----------------------96.0-|             +100.0-|
                     |      |                                  |      +-34.0-|      +------D.bif-12S
                     |      |                                  |      |      |
                     |      |                                  +-30.0-|      +-------------A.gen-12S
              +-93.0-|      |                                         |
              |      |      |                                         +--------------------A.itoi-12S
              |      |      |
              |      |      |                                                       +------mz-bud17
       +-92.0-|      |      +-------------------------------------------------100.0-|
       |      |      |                                                              +------mt-bud37
       |      |      |
+------|      |      +---------------------------------------------------------------------mp-sp68
|      |      |
|      |      +----------------------------------------------------------------------------mj-sp58
|      |
|      +-----------------------------------------------------------------------------------mz-sp18
|
+------------------------------------------------------------------------------------------mm-sp28
```

This is an unrooted tree

Figure 25. Bootstrap analysis of 12S gene neighbor-joining tree by using Phylip 3.6

This neighbor-joining tree for gall-midges of *Machilus* is based on average 430 bp of the mitochondrial 12s gene and Kimura's two-parameter distance. There are 5 out-group sequences and 28 sample sequences.

Unknown gall midge species are named after host plant, gall type, serial number in Table 1 and Table 2. Letters before dash line are host plane abbreviation. **1.MZ :** *Machilus zuihensis Hayata.* **2.MM:** *Machilus zuihoensis. var. mushaensis.* **3.MT**: *Machilus thunbergii.* **4.MJK:** *Machilus japonica kusanoi.* **5.MJ:** *Machilus japonica.* **6.MP:** *Machilus philippinense.* **7.MO:** *Machilus obovatifolia.* ***8.*MK**: *Machilus konishii Hayata.* Letters after dash line are gall types.1.mice. 2.bell. 3.bird. 4.club. 5.bulb. 6.blister. 7.bud. 8.spindle. 9.bullet. The dots in each sketch represent how many larvae are in one gall. One dot means there is only one larva in one gall. Three dots represent that there are more than one larva in one gall. Numbers beside sketches are larva types in table 5.

Since the maxium number of sample name is 10 charecters in phylip program, there are some shortence of some sample name. "Bullet" is shortened to "bu". "Spindle" is shortened to "sp". The name of 5 outgroups are also shortened.

Consensus tree program, version 3.67

Species in order:

1. mm-sp28
2. mz-bud17
3. mt-bud37
4. D.bif-12S
5. D.amb-12S
6. A.gen-12S
7. A.sph-12S
8. A.itoi-12S
9. mt-mice31
10. mjk-mice41

11. mm-bulb25
12. mz-bulb15
13. mj-bulb55
14. mm-mice21
15. mz-mice11
16. mj-mice51
17. mm-bell22
18. mt-bell32
19. mjk-bell42
20. mp-club64

21. mj-club54
22. mz-bell12
23. mo-club74
24. mt-club34
25. mj-bu59
26. mt-bu39
27. mjk-bu49
28. mp-bu69
29. mo-bu79
30. mm-bu29

31. mz-bu19

32. mp-sp68
33. mj-sp58
34. mz-sp18

Sets included in the consensus tree

Set (species in order)   How many times out of   100.00

....**.... ....   100.00
....***.... ....   100.00
....***. 100.00
....* *...   100.00
...**..... ....   100.00
.**....... ....   100.00
....**** ****...... ....   100.00
....***. 100.00
........** 100.00
.********* ********** ********** *...   100.00
....**.... ....   99.00
...******* ********** ********** *...   96.00
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.********* ********** ********** **..   93.00
.********* ********** ********** ***.   92.00
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........** ***....... ....   71.00
....******* ********** *...   69.00
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....* *......... ....   62.00
....**** **........ ....   52.00
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...***.*.. ....   30.00

Sets NOT included in consensus tree:

Set (species in order)   How many times out of   100.00

.... *.*....... ....   44.00
...**..*.. ....   31.00
...**.**.. ....   29.00
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....**** **.*...... ....   27.00
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Appendix 2. COI Gene Sequence Alignment

```
                    Symbol comparison table: pileupdna.cmp  CompCheck: 6876

              GapWeight: 5
        GapLengthWeight: 1

mz-bel12_pileup_12489.txt  MSF: 536  Type: N  June 7, 2007 18:10  Check: 1087 ..

Name: Asphondylia_sphaera-Asphodyliini  Len:   536  Check:  637  Weight:  1.00
Name: Asphondylia_gennadii-Asphodyliini  Len:   536  Check:  911  Weight:  1.00
Name: Asphondylia_itoi-Asphodyliini  Len:   536  Check: 5604  Weight:  1.00
Name: mj-blister56     Len:   536  Check: 6062  Weight:  1.00
Name: mo-blister76     Len:   536  Check: 2456  Weight:  1.00
Name: mt-blister36     Len:   536  Check: 3362  Weight:  1.00
Name: mjk-bulb45       Len:   536  Check: 4371  Weight:  1.00
Name: mj-bulb55        Len:   536  Check: 2505  Weight:  1.00
Name: mm-bulb25        Len:   536  Check: 5539  Weight:  1.00
Name: mt-bullet39      Len:   536  Check: 4124  Weight:  1.00
Name: mj-bullet59      Len:   536  Check: 4340  Weight:  1.00
Name: mz-bullet19      Len:   536  Check: 7475  Weight:  1.00
Name: mm-bullet29      Len:   536  Check: 4713  Weight:  1.00
Name: mo-bullet79      Len:   536  Check: 4503  Weight:  1.00
Name: mjk-bullet49     Len:   536  Check: 7592  Weight:  1.00
Name: mp-bullet69      Len:   536  Check: 7579  Weight:  1.00
Name: mj-mice51        Len:   536  Check: 7909  Weight:  1.00
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Name: mjk-mice41 Len: 536 Check: 1996 Weight: 1.00
Name: mt-mice31 Len: 536 Check: 6747 Weight: 1.00
Name: mz-mice11 Len: 536 Check: 2413 Weight: 1.00
Name: mm-mice21 Len: 536 Check: 8856 Weight: 1.00
Name: mt-bell32 Len: 536 Check: 5561 Weight: 1.00
Name: mm-bell22 Len: 536 Check: 5775 Weight: 1.00
Name: mjk-bell42 Len: 536 Check: 4717 Weight: 1.00
Name: mt-club34 Len: 536 Check: 4772 Weight: 1.00
Name: mo-club74 Len: 536 Check: 4233 Weight: 1.00
Name: mp-club64 Len: 536 Check: 7827 Weight: 1.00
Name: mz-bell12 Len: 536 Check: 7820 Weight: 1.00
Name: mk-bird83 Len: 536 Check: 7944 Weight: 1.00
Name: mj-club54 Len: 536 Check: 7085 Weight: 1.00
Name: mjk-club44 Len: 536 Check: 4477 Weight: 1.00
Name: mp-spindle68 Len: 536 Check: 1892 Weight: 1.00
Name: mt-spindle38 Len: 536 Check: 7329 Weight: 1.00
Name: mm-spindle28 Len: 536 Check: 7015 Weight: 1.00
Name: mz-spindle18 Len: 536 Check: 3732 Weight: 1.00
Name: mj-spindle58 Len: 536 Check: 2869 Weight: 1.00
Name: mz-bud17 Len: 536 Check: 9820 Weight: 1.00
Name: mt-bud37 Len: 536 Check: 8525 Weight: 1.00

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1 50

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Asphondylia_ tcgtttaaat aatataagat tttgactatt acctccatca ttaactattt
Asphondylia_ tcgccttaat aatataagat tttgactttt acctccatca ttaacaattt
Asphondylia_ tcgattaaat aatataagat tttgacttct tcctccatca ttaactatcc
mj-blister56 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~TCAATTAT
mo-blister76 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GTGT GATCAAAACA
mt-blister36 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~CTTAAG
  mjk-bulb45 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~C AAAACATATA
   mj-bulb55 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~CAT TCTACACTAT
   mm-bulb25 ~~~~~TTACA TAAATTATAT TTCCCCAGGG TATGTTTTTT TTTATTTTTA
 mt-bullet39 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~CTATT AAGACCATCA CTTTTAATAA
 mj-bullet59 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~G GGTTATTTTT TTGTTTCACC
 mz-bullet19 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~CT ACCACCATCA ATTCTAATTA
 mm-bullet29 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGGCG ACCACCATCA ATTCTAATTA
 mo-bullet79 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~CTTATT ACCTCCATCA ATTTTAATTC
mjk-bullet49 ~~~~~~~~~~ ~~~~~~TTTA GTTTCGGGTT TCCTCCATCA ATTTTAATTC
 mp-bullet69 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~GG TTCCTCCTCA ATTTTAATTC
   mj-mice51 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~ATT ACCTCCTTCA CTTTCTATTC
  mjk-mice41 ~~~~~~~~~~ ~~~~~~~~~~ ~~~GATTATT ACCTCCTTCA CTTTCTATTC
   mt-mice31 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~TATT ACCTCCTTCA CTTTCTATTC
   mz-mice11 ~~~~~~~~~~ ~~~~~~TTAG ATCCGAGAGG ACCTCCTTCA CTTTCTATTC
   mm-mice21 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~CTTT ACCTCCATCA CTTTCTATTC
   mt-bell32 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~TTACT ACCTCCATCA TTATCTATTC
   mm-bell22 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GGCT ACCTCCATCA TTATCTATTC
  mjk-bell42 ~~~~~~~~~~ ~~~~~~~TAG ATTTGATTCT ACCTCCATCA TTATCTATTC
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   mt-club34 ~~~~~~~~~~ ~~~TATTAAT TTTCCGGGGA ACCTCCATCA TTATCTATTC
   mo-club74 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ACCTCCATCA TTATCTATTC
   mp-club64 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ACCTCCTTCA TTGTCTATTC
   mz-bell12 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GGAT ACCTCCATCA CTATCTATTC
   mk-bird83 ~~~~~~~~~~ ~~~~~~~~~T ACACGGGGCT ACCTCCATCA CTATCTATTC
   mj-club54 ~~~~GGCTAT TTGTAGTATT ATCGAGGACT ACCTCCTTCA TTATCTATTC
  mjk-club44 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~T TTATCTATTC
mp-spindle68 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~CCCCCTCT TTACCTTATT
mt-spindle38 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~CTT TAACCTTATT
mm-spindle28 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~AA AAATTTTATT
mz-spindle18 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~AATTTTATT
mj-spindle58 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~TT TAATTTTATT
    mz-bud17 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~TTC AAACTTTATA
    mt-bud37 ~~~~~~~~~~ ~~~~TTTTAA ATACGGGTCT CCCTCCTTCT TTAATATTAC

             51                                               100
Asphondylia_ tattaataag aagaattatt .gaaaacggg actggaaccg gatgaactat
Asphondylia_ tattaataag aagaattatt .gaaagaggg acaggaacag gatgaacagt
Asphondylia_ tattaataag ttcaattatc .gaaagaggg acaggaacag gctgaacaat
mj-blister56 CTAATTAAGA ATGATTAATA AAAACTGGGA CTGGGGACTG GATGAACAGT
mo-blister76 TTAATTAAGA AGAATAATAG AAACTTGGAA CTAGGAACTG GATGAACAGT
mt-blister36 GCATTTGAAG GGCAGCACAA CAGGCGAATC AGGAACATGG TTCGAACAGT
   mjk-bulb45 TTATATAAAG AAAAACTGGT AAAAACAGGA ACAGGAACGG GATGAACTGT
    mj-bulb55 GTTATTAAAG AAAAACTTGT AAAAACAGGA ACAGGAACGG GATGAACTGT
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mm-bulb25	CCCGGGGATT	TGAATTCTGT	AGAAACAGGA	ACAGGGACAG	GATGAACTGT
mt-bullet39	TTATTTTAAG	AATAATAATT	.GAAATAGGA	ACTGGAACAG	GATGAACTAT
mj-bullet59	GGGTTTTAAG	AATAATAGGG	.GAAAGAGGA	GCTGGAACAG	GATGAACTAT
mz-bullet19	TTCTTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACTAT
mm-bullet29	TTCTTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACTAT
mo-bullet79	TTCTTATAAG	AAGAATAATT	.GAAAAAGGA	ACTGGAACAG	GATGAACTGT
mjk-bullet49	TTCTTATAAG	AAGAATAATT	.GAAAAAGGA	ACTGGAACAG	GATGAACTAT
mp-bullet69	TTCTTATAAG	AAGAATAATT	.GAAAAAGGA	ACTGGAACAG	GATGAACTAT
mj-mice51	TTCTTTTAAG	AAGAATAATT	.GAAAGGGGA	ACTGGAACAG	GATGAACTGT
mjk-mice41	TTCTTTTAAG	AAGAATAATT	.GAAAGGGGA	ACTGGAACAG	GATGAACTGT
mt-mice31	TTCTTTTAAG	AAGAATAATT	.GAAAGGGGA	ACTGGAACAG	GATGAACTGT
mz-mice11	TTCTTTCAAG	AAGAATAATT	.GAAAGGGGA	ACTGGAACAG	GATGAACTGT
mm-mice21	TTCTTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACAGT
mt-bell32	TTATTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACAGT
mm-bell22	TTATTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACAGT
mjk-bell42	TTATTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACAGT
mt-club34	TTATTATAAG	AAGAATAATT	.GAAAGAGGC	ACTGGAACAG	GATGAACAGT
mo-club74	TTATTATAAG	AAGAATAATT	.GAAAGAGGC	ACTGGAACAG	GATGAACAGT
mp-club64	TTATTTTAAG	AAGAATAATT	.GAAAGAGGT	ACTGGAACAG	GATGAACAGT
mz-bell12	TTATTTTAAG	AAGAATAATT	.GAAAGAGGT	ACTGGAACAG	GATGAACAGT
mk-bird83	TTATTTTAAG	AAGAATAATT	.GAAAGAGGT	ACTGGAACAG	GATGAACAGT
mj-club54	TTATTTTAAG	AAGAATAATT	.GAAAGAGGT	ACTGGAACAG	GATGAACAGT
mjk-club44	TTATTTTAAG	AAGAATAATT	AGAAAGAGGA	CTAGGAACAG	GATGAACAGT
mp-spindle68	ATTAATT.AG	AAGAATTATT	.GAAAATGGA	ACAGGAACAG	GATGAACAAT

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mt-spindle38 ATTAATT.AG AAGAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
mm-spindle28 ATTAA.TAAG AATAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
mz-spindle18 ATTAA.TCAG AATAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
mj-spindle58 ATTAATTCAG AATAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
     mz-bud17 TATTAATTAA AAGAATAGTA .GAAACTGGA TCAGGAACAG GATGAACTAT
     mt-bud37 TTTTAATCAG AAGAAT.GGT AGAAACAGGG ACAGGGACAG GATGAACTGT

             101                                               150
Asphondylia_ ttatcctcct ttatcatcaa ttattgctca taatggaaga tcaactgatt
Asphondylia_ ttatcccccct ttatcctcaa ttattgctca taatagaaga tcaacagatt
Asphondylia_ ttacccccca ttatcatcta ttattgctca taatagaaga tcaactgatt
 mj-blister56 TTATCCCCCT CTTTCATCAA CAATTGCTCA TACTGGATCT TCAGTATATT
 mo-blister76 CTATCCACCC CTTTCTTCTA TTATTGCACA TACAGGCTCT TCTGTAGATT
 mt-blister36 TTACCCTCCA CTTTCATCAA CTATTGCTCA TACAGGATCA TCTGTTGATT
   mjk-bulb45 ATATCCACCA CTTTCATCAA TTATTGCCCA TAATGGAGCA TCTGTTGACT
    mj-bulb55 ATATCCACCA CTTTCATCAA TTATTGCCCA TAATGGAGCA TCTGTTGACT
    mm-bulb25 ATACCCACCA CTCTCATCAA TTATTGCCCA TAATGGTGCG TCTGTTGACT
  mt-bullet39 TTATCCCCCT CTTTCTTCAA TTATAGCACA TAGTAGAGCA TCTGTAGATT
  mj-bullet59 TTACCCCCCT CTTTCTTCAA TTATAGCACA TAAGGGAGCA TCTGTAGACT
  mz-bullet19 TTATCCACCT CTTTCCGCAA TTATTGCACA TAATAGACCA TCTGTTGATC
  mm-bullet29 TTATCCGCCT CTTTCCGCAA TTATTGCACA TAATAGACCA TCTGTTGATC
  mo-bullet79 TTACCCCCCT CTATCTTCAA TTATTGCGCA TAATGGACCA TCTGTTGATC
 mjk-bullet49 TTACCCCCCT CTATCTTCAA TTATTGCACA TAATGGACCA TCTGTTGATC
  mp-bullet69 TTACCCTCCT CTATCTTCAA TTATTGCACA TAATGGACCA TCTGTTGATC
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mj-mice51 CTACCCACCT CTTTCTTCTA TTATAGCCCA TAATAGATCA TCAGTAGATT
mjk-mice41 CTACCCACCT CTTTCTTCTA TTATAGCCCA TAATAGATCA TCAGTAGATT
mt-mice31 CTACCCACCT CTTTCTTCTA TTATAGCCCA TAATAGATCA TCAGTAGATT
mz-mice11 CTACCCACCT CTTTCTTCTA TTATAGCCCA TAATAGATCA TCAGTAGATT
mm-mice21 CTACCCACCT CTTTCTTCTA TTATAGCCCA TAATAGATCA TCAGTAGATT
mt-bell32 ATACCCTCCT CTTTCTTCAA TTATAGCGCA TAATGGATCA TCAGTAGATT
mm-bell22 ATACCCTCCT CTTTCTTCAA TTATAGCGCA TAATGGATCA TCAGTAGATT
mjk-bell42 ATACCCTCCT CTTTCTTCAA TTATAGCGCA TAATGGATCA TCAGTAGATT
mt-club34 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
mo-club74 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
mp-club64 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCG TCAGTAGATT
mz-bell12 TTACCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
mk-bird83 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
mj-club54 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
mjk-club44 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
mp-spindle68 TTACCCTCCT CTTTCTTCAA TTATTGCACA TACTAGACCT TCAGTAGACT
mt-spindle38 TTACCCTCCT CTTTCTTCAA TTATTGCACA TACTAGACCT TCAGTAGACT
mm-spindle28 TTATCCCCCC CTTTCTTCTA TTACTGCACA TACTAGAAAT TCAGTAGATT
mz-spindle18 TTATCCCCCC CTTTCTTCTA TTAATGCACA TACTAGAAAT TCAGTAGATT
mj-spindle58 TTACCCCCCC CTTTCTTCTA TTACTGCACA TACTAGAACT TCAGTAGATT
mz-bud17 TTATCCCCCC TTATCTTCCA TTATTGCTCA TACAAGTTCT TCAGTAGATT
mt-bud37 ATATCCTCCT CTTTCTTCAT CAATTGCCCA TACTGGCTCA TCAGTTGATT

151 200

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Asphondylia_ tatcaatctt ttcacttcat atcgcaggaa tttcttctat tttaggagct
Asphondylia_ tatctatttt ttcacttcat attgctggta tctcttctat tttaggagct
Asphondylia_ tatcaatttt ttcattacac attgcaggaa tctcttctat tttaggggca
mj-blister56 TTTCTATTTT TTCTCTTCAT ATTGCTGGAA TTTCTTCTAT TTTAGGAGCA
mo-blister76 TTTCAATTTT TTCACTACAT ATCGCGGGAA TCTCATCCAT TTTGGGGGCG
mt-blister36 TTTCTATTTT TTCACTACAT ATTGCAGGAA TTTCATCAAT CCTAGGAGCA
  mjk-bulb45 TATCATTTTT TTCTCTTCAT ATTGCAGGAA TTTCATCATT TTTAGGAGCA
   mj-bulb55 TATCATTTTT TTCTCTTCAT ATTGCAGGAA TTTCATCATT TTTAGGAGCA
   mm-bulb25 TATCAATTTT TTCTCTCCAT ATTGCAGGAA TCTCATCAAT TTTGGGAGCA
 mt-bullet39 TATCTATTTT TTCACTTCAT ATAGCAGGAA TTTCATCAAT TTTAAGATCT
 mj-bullet59 TATCTATTTT TTCACTTCAT ATAGCAGGAA TTTCATCAAT TTTAAGATCT
 mz-bullet19 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
 mm-bullet29 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
 mo-bullet79 TATCTATTTT TTCACTACAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
mjk-bullet49 TTTCTATTTT TTCACTACAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
 mp-bullet69 TATCTATTTT TTCACTACAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
   mj-mice51 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT CTTAGGAGCT
  mjk-mice41 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT CTTAGGAGCT
   mt-mice31 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT CTTAGGAGCT
   mz-mice11 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT CTTAGGAGCT
   mm-mice21 TATCTATTTT TTCGCTTCAT ATTGCAGGAA TTTCATCAAT CTTAGGAGCT
   mt-bell32 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCA
   mm-bell22 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCA
  mjk-bell42 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCA
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     mt-club34 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
     mo-club74 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
     mp-club64 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
      mz-bell12 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
     mk-bird83 TATCTATTTT TTCCCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
     mj-club54 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
    mjk-club44 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
  mp-spindle68 TCTCTATTTT TTCCCTTCAT ATTGCTGGAA TTTCCTCTAT CTTAGGAGCA
  mt-spindle38 TCTCTATTTT TTCCCTTCAT ATTGCTGGAA TTTCCTCTAT CTTAGGAGCA
  mm-spindle28 TTTCAATTAT TTCCCTCCAT ATAGCTGGAA TTTCCTCTAT TTTAGGAGCA
  mz-spindle18 TTTCAATTAT TTCCCTCCAT ATAGCTGGAA TTTCCTCTAT TTTAGGAGCA
  mj-spindle58 TTTCTATTTT TTCCCTCCAT ATTGCTGGAA TTTCCTCTAT TTTAGGAGCA
      mz-bud17 TTTCATTTTT TTCACTTCAT ATAGCTGGAA TTTCTTCTAT TTTAGGAGCT
      mt-bud37 TCTCAATTTT TTCTTTACAT ATTGCTGGTA TTTCCTCAAT TTTAGGGGCA

               201                                              250
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   Asphondylia_ attaatttta ttactactat cattaatata aaaaataaat ttattaaatt
   Asphondylia_ attaatttta ttactacaat tattaatata aaaaataaat ttattaaatt
   mj-blister56 ATTAATTTTA TTTCAACTAT ATTTAATATA AAAATTAAAT TTTTAAAATT
   mo-blister76 ATTAATTTTA TTTCAACTAT ATTAAATATA AAAATTAAAT TTTTAAAATT
   mt-blister36 ATTAATTTTA TTTCAACTAT ATTTAATATA AAAATTAAAT TTTTAAAATT
     mjk-bulb45 ATTAATTTTA TTTCAACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
      mj-bulb55 ATTAATTTTA TTTCAACTAT TATAAATATA AAAAATAAAA ATTTAAAATT
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mm-bulb25 ATTAATTTTA TCTCAACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mt-bullet39 ATAAATTTTA TTTCTACAAT TATAAATATA AAAAATATAA ATTTAAAATT
mj-bullet59 ATCAATTTTA TTTCTACAAT TATAAATATA AAAAATATAA ATTTAAAATT
mz-bullet19 ATCAATTTTA TTTCTACAAT TATAAATATA AAAAATAATA ATTTAAATTT
mm-bullet29 ATCAATTTTA TTTCTACAAT TATAAATATA AAAAATAATA ATTTAAATTT
mo-bullet79 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAATA ATTTAAAATT
mjk-bullet49 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAATA ATTTAAAATT
mp-bullet69 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAATA ATTTAAAATT
mj-mice51 ATTAATTTTA TTTCTACGAT TATAAATATA AAAAACAAAA ATTTAAAATT
mjk-mice41 ATTAATTTTA TTTCTACGAT TATAAATATA AAAAACAAAA ATTTAAAATT
mt-mice31 ATTAATTTTA TTTCTACGAT TATAAATATA AAAAACAAAA ATTTAAAATT
mz-mice11 ATTAATTTTA TTTCTACGAT TATAAATATA AAAAACAAAA ATTTAAAATT
mm-mice21 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mt-bell32 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mm-bell22 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mjk-bell42 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mt-club34 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mo-club74 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mp-club64 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mz-bell12 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mk-bird83 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mj-club54 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mjk-club44 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mp-spindle68 ATTAATTTTA TTTCAACAAT AATAATATA AAAATTAAAT TTCTAAAGAT

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mt-spindle38 ATTAATTTTA TTTCAACAAT AATTAATATA AAAATTAAAT TTCTAAAGAT
mm-spindle28 ATTAATTTTA TCTCAACAAT AATTAATATA AAAATTAAAT ATTTAAAAAT
mz-spindle18 ATTAATTTTA TCTCAACAAT AATTAATATA AAAATTAAAT ATTTAAAAAT
mj-spindle58 ATTAATTTTA TCTCAACAAT AATTAATATA AAAATTAAAT ATTTAAAAAT
     mz-bud17 ATTAATTTTA TTTCTACTAT TTTAAATATA AAAATTAAAT TTATTAAAGC
     mt-bud37 ATTAACTTTA TTTCAACAAT ACTAAATATA AAAATTAAAT TTATTAAATT

             251                                              300
Asphondylia_ taatgaatta tcactttttta tctgatcaat tttaattact accattcttt
Asphondylia_ aaatgaactt tccctttttta tttgatcaat tttaattact actgttcttt
Asphondylia_ taatgaaata tcactattta tttgatcaat tctaattaca actattcttt
mj-blister56 CGATCAAATT TCTTTATTTA CATGATCAGT ACTAATTACA GCATTTTTAT
mo-blister76 TGATCAAATT TCTTTATTTA TTTGATCTAT TATAATCACT ACTATCCTTT
mt-blister36 TGACCAAATT TCTTTATTTA CATGATCAGT ATTAATTACT GCATTTCTTT
   mjk-bulb45 TAATAAATTA TCTTTATTTA TTTGATCAAT TTTAATTACA ACTATTTTAT
    mj-bulb55 TAATAAATTA TCTTTATTTA TTTGATCAAT TTTAATTACA ACTATTTTAT
    mm-bulb25 TAATAAATTA TCTTTATTTA TTTGATCAAT TTTAATTACA ACTATTTTAT
  mt-bullet39 TTATGAACTT TCTTTATTCA TTTGATCAAT TCTCATTACA TCAATTTTAT
  mj-bullet59 TTATGAACTT TCTTTATTTA TTTGATCAAT TCTTATTACA TCAATTTTAT
  mz-bullet19 TAATAAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
  mm-bullet29 TAATAAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
  mo-bullet79 TAATAAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
 mjk-bullet49 TAATAAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
  mp-bullet69 TAATAAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
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    mj-mice51 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATCACA ACAATTTTAT
   mjk-mice41 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATCACA ACAATTTTAT
    mt-mice31 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATCACA ACAATTTTAT
    mz-mice11 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATCACA ACAATTTTAT
    mm-mice21 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
    mt-bell32 TAATGAACTT TCTTTATTCA TTTGATCAAT TTTTATTACA ACAATCTTAT
    mm-bell22 TAATGAACTT TCTTTATTCA TTTGATCAAT TTTTATTACA ACAATCTTAT
   mjk-bell42 TAATGAACTT TCTTTATTCA TTTGATCAAT TTTTATTACA ACAATCTTAT
    mt-club34 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
    mo-club74 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
    mp-club64 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATTACT ACAATTTTAT
    mz-bell12 TAATGAACTT TCTTTATTCA TTTGATCAAT TTTTATTACT ACAATTTTAT
    mk-bird83 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
    mj-club54 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATTACT ACAATTTTAT
   mjk-club44 TAATGAACTT TCTTTATTTA TTTGATCAAT TTTTATTACT ACAATTTTAT
 mp-spindle68 AGATCAAATA TCATTATTTA TTTGATCTAT TTTAATTACA ACGATCTTAT
 mt-spindle38 AGATCAAATA TCATTATTTA TTTGATCTAT TTTAATTACA ACAATCTTAT
 mm-spindle28 AGATCAAATA TCACTTTTTA TTTGATCTAT TTATATTACA ACAATTCTTT
 mz-spindle18 AGATCAAATA TCACTTTTTA TTTGATCTAT TTATATTACA ACAATTCTTT
 mj-spindle58 AGATCAAATA TCATTTTTTA TTTGATCTAT TTATATTACA ACAATTCTTT
     mz-bud17 TAATCAAATT TCATTATTTA TTTGATCAAT TATTATTACA GCTATTTTAC
     mt-bud37 TGATCAAATT TCTTTATTCA CATGATCAGT ATTAATTACT GCCATTCTTT

              301                                              350
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Asphondylia_ tacttttatc tttaccagtt cttgcaggag caatcactat actaattact
Asphondylia_ tacttttatc acttcctgta cttgcaggag caattactat attattaact
Asphondylia_ tattattatc attacctgta ttagctggag caattactat attattaact
mj-blister56 TATTATTATC ATTACCAGTT TTAGCTGGAG CTATTACAAT ATTATTAATA
mo-blister76 TACTCCTTTC TTTACCTATT TTAGCAGGAG CTATTACTAT ACTTTTAACT
mt-blister36 TATTATTATC TTTACCAGTA TTAGCAGGAG CAATTACAAT ATTATTAATA
  mjk-bulb45 TACTTTTATC ATTACCTGTT TTGGCCGGAG CTATTACAAT ATTATTAACA
   mj-bulb55 TACTTTTATC ATTACCTGTT TTGGCCGGAG CTATTACAAT ATTATTAACA
   mm-bulb25 TACTTTTATC ATTACCTGTT TTGGCCGGAG CTATTACAAT ATTATTAACA
 mt-bullet39 TATTACTATC ATTACCAGTC TTAGCTGGAG CAATTACAAT ATTATTAACT
 mj-bullet59 TATTACTATC ATTACCAGTA TTAGCTGGAG CAATCACAAT ATTATTAACT
 mz-bullet19 TATTACTATC CTTACCAGTT TTAGCTGGAG CAATCACAAT ATTGCTAACT
 mm-bullet29 TATTACTATC CTTACCAGTT TTAGCTGGAG CAATCACAAT ATTACTAACT
 mo-bullet79 TATTATTATC TTTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACT
mjk-bullet49 TATTATTATC TTTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACT
 mp-bullet69 TATTATTATC TTTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACT
   mj-mice51 TATTATTATC TTTACCAGTA TTAGCTGGAG CAATTACAAT ATTATTAACT
  mjk-mice41 TATTATTATC TTTACCAGTA TTAGCTGGAG CAATTACAAT ATTATTAACT
   mt-mice31 TATTATTATC TTTACCAGTA TTAGCTGGAG CAATTACAAT ATTATTAACT
   mz-mice11 TATTATTATC TTTACCAGTA TTAGCTGGAG CAATTACAAT ATTATTAACT
   mm-mice21 TATTATTATC TTTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACT
   mt-bell32 TATTATTATC ATTACCAGTT TTAGCAGGAG CAATTACAAT ATTATTAACA
   mm-bell22 TATTATTATC ATTACCAGTT TTAGCAGGAG CAATTACAAT ATTATTAACA
  mjk-bell42 TATTATTATC ATTACCAGTT TTAGCAGGAG CAATTACAAT ATTATTAACA
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mt-club34 TATTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACA

mo-club74 TATTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACA

mp-club64 TATTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACA

mz-bell12 TGTTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACA

mk-bird83 TATTACTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACA

mj-club54 TATTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ACTATTAACA

mjk-club44 TATTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ACTATTAACA

mp-spindle68 TAATTATTTC TTTACCTGTT TTAGCAGGAG CAATTACTAT ATTATTAACA

mt-spindle38 TAATTATTTC TTTACCTGTT TTAGCAGGAG CAATTACTAT ATTATTAACA

mm-spindle28 TATTACTTGC ATTACCTGTT TTAGCAGGAG CAATTACTAT ACTACTAACA

mz-spindle18 TATTACTTGC ATTACCTGTT TTAGCAGGAG CAATTACTAT ACTACTAACA

mj-spindle58 TATTACTTGC TTTACCTGTT TTAGCAGGAG CAATTACTAT ACTACTAACA

mz-bud17 TTCTTCTTTC ATTACCTGTT TTAGCTGGAG CAATTACTAT ATTATTAACT

mt-bud37 TATTATTATC ACTACCAGTT CTAGCCGGAG CAATTACTAT ACTTCTTACT

351 400

Asphondylia_ gaccgaaact taaatacttc atttttttgat cctataggag ggggtgaccc

Asphondylia_ gatcgaaata ttaatactac attttttgac ccaataggag gaggagatcc

Asphondylia_ gatcgaaata ttaatacatc tttttttgat cctataggag ggggtgatcc

mj-blister56 GATCGAAACT TAAATACATC ATTCTTTGAT CCTATAGGAG GAGGAGATCC

mo-blister76 GATCGAAATT TAAATACATC ATTTTTTGAC CCCATAGGAG GAGGAGATCC

mt-blister36 GACCGTAATT TAAATACATC ATTTTTTGAT CCAATAGGAG GAGGAGATCC

mjk-bulb45 GATCGAAATA TAAATACATC TTTTTTTGAC CCACTCGGAG GAGGAGATCC

mj-bulb55 GATCGAAATA TAAATACATC ATTTTTTGAC CCACTCGGAG GAGGAGATCC mm-bulb25 GATCGAAATA TAAATACATC ATTTTTTGAC CCACTCGGAG GAGGAGACCC

mt-bullet39 GATCGAAATT TAAATACTTC TTTTTTTGAT CCTATTGGAG GAGGAGATCC

mj-bullet59 GATCGAAATT TAAATACTTC TTTTTTTGAT CCTATTGGAG GAGGTGATCC

mz-bullet19 GATCGAAATT TAAATACATC TTTTTTCGAT CCATTAGGAG GTGGAGATCC

mm-bullet29 GATCGAAATT TAAATACATC TTTTTTCGAT CCATTAGGAG GTGGAGATCC

mo-bullet79 GATCGAAATT TAAATACATC TTTCTTTGAC CCATTAGGAG GTGGAGATCC

mjk-bullet49 GATCGAAATT TAAATACATC TTTCTTTGAC CCATTAGGAG GTGGAGATCC

mp-bullet69 GATCGAAATT TAAATACATC TTTCTTTGAC CCATTAGGAG GTGGAGATCC

mj-mice51 GATCGAAATT TAAATACTTC ATTTTTTGAC CCACTAGGAG GAGGAGATCC

mjk-mice41 GATCGAAATT TAAATACTTC ATTTTTTGAC CCACTAGGAG GAGGAGATCC

mt-mice31 GATCGAAATT TAAATACTTC ATTTTTTGAC CCACTAGGAG GAGGAGATCC

mz-mice11 GATCGAAATT TAAATACTTC ATTTTTTGAC CCACTAGGAG GAGGAGATCC

mm-mice21 GATCGAAATT TAAATACTTC ATTTTTTGAT CCTCTAGGAG GGGGAGATCC

mt-bell32 GATCGAAATT TAAATACTTC TTTTTTTGAT CCATTAGGAG GAGGAGATCC

mm-bell22 GATCGAAATT TAAATACTTC TTTTTTTGAT CCATTAGGAG GAGGAGATCC

mjk-bell42 GATCGAAATT TAAATACTTC TTTTTTTGAT CCATTAGGAG GAGGAGATCC

mt-club34 GATCGAAATT TAAACACTTC TTTTTTTGAT CCATTAGGAG GAGGAGATCC

mo-club74 GATCGAAATT TAAACACTTC TTTTTTTGAT CCATTAGGAG GAGGAGATCC

mp-club64 GATCGAAATT TAAACACTTC TTTTTTTGAT CCATTAGGAG GAGGAGATCC

mz-bell12 GATCGAAATT TAAATACTTC TTTTTTTGAT CCATTAGGAG GAGGAGATCC

mk-bird83 GATCGAAATT TAAATACTTC TTTTTTTGAT CCATTAGGAG GAGGAGATCC

mj-club54 GATCGAAATT TAAACACTTC TTTTTTTGAT CCATTAGGAG GAGGAGATCC

mjk-club44 GATCGAAATT TAAACACTTC TTTTTTTGAT CCATTAGGAG GAGGAGATCC

mp-spindle68 GATCGAAATC TAAACACATC ATTTTTCGAT CCTATAGGAG GAGGAGATCC

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mt-spindle38 GATCGAAATC TAAACACATC ATTTTTCGAT CCTATAGGAG GAGGAGATCC
mm-spindle28 GATCGAAATC TAAATACATC ATTTTTTGAC CCAATAGGAG GAGGAGATCC
mz-spindle18 GATCGAAATC TAAATACATC ATTTTTTGAC CCAATAGGAG GAGGAGATCC
mj-spindle58 GATCGAAATC TAAATACATC ATTTTTTGAC CCAATAGGAG GAGGAGATCC
     mz-bud17 GATCGAAATT TAAATACATC ATTTTTTGAT CCTATAGGAG GAGGTGACCC
     mt-bud37 GATCGAAATT TAAATACATC TTTTTTTGAT CCAATAGGGG GAGGAGATCC

             401                                              450
Asphondylia_ aattctttat caacatttat tttgattttt tggtcatcct ~~~~~~~~~~
Asphondylia_ aattctttat caacatttat tttgattttt tggtcatcct ~~~~~~~~~~
Asphondylia_ tattctttat caacatttat tttgattttg cggtcaccca ~~~~~~~~~~
mj-blister56 AATTTTATAT CAACATTTAT TTTGATTTTT TGGACATCCA GAATTTTATA
mo-blister76 ATTTTTATAT CAACACTTAT TTTGATTTTT TGGCCACCCT GAATTTTATA
mt-blister36 AATTTTATAT CAACATTTAT TTTGATTTTT TGGTCACCCA GAAGTTTATA
   mjk-bulb45 AATTTTATAT CAACATTTAT TTTGATTTTT TGGACATCCA GAATTTTATA
    mj-bulb55 AATTTTATAT CAACATTTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
    mm-bulb25 AATTTTATAC CAACATTTAT TTTGATTTTT TGGACACCCA GAAGTTTATA
  mt-bullet39 TATTCTATAT CAACATCTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
  mj-bullet59 TATTCTTTAT CAACATCTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
  mz-bullet19 AATTTTATAT CAACATTTAT TTTGATTTTT TGGCCACCCA GAAGTTTATA
  mm-bullet29 AATTTTATAT CAACATTTAT TTTGATTTTT TGGTCACCCA GAAGTTTATA
  mo-bullet79 AATTTTATAT CAACATTTAT TTTGATTTTT TGGTCATCCA GAAGTTTATA
 mjk-bullet49 AATTTTATAT CAACATTTAT TTTGATTTTT TGGTCATCCA GAAGTTTATA
  mp-bullet69 AATTTTATAT CAACATTTAT TTTGATTTTT TGGTCATCCA GAAGTTTATA
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     mj-mice51 AATTCTTTAT CAACATTTAT TTTGATTCTT TGGACATCCT GAAGTTTATA
    mjk-mice41 AATTCTTTAT CAACATTTAT TTTGATTCTT TGGACATCCT GAAGTTTATA
     mt-mice31 AATTCTTTAT CAACATTTAT TTTGATTCTT TGGACATCCT GAAGTTTATA
     mz-mice11 AATTCTTTAT CAACATTTAT TTTGATTCTT TGGACATCCT GAAGTTTATA
     mm-mice21 AATTCTTTAT CAACATTTAT TTTGATTCTT TGGACATCCT GAAGTTTATA
     mt-bell32 AATTCTTTAT CAACATTTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
     mm-bell22 AATTCTTTAT CAACATTTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
    mjk-bell42 AATTCTTTAT CAACATTTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
     mt-club34 AATTCTTTAT CAACATTTAT TCTGATTTTT TGGGCATCCA GAAGTTTATA
     mo-club74 AATTCTTTAT CAACATTTAT TCTGATTTTT TGGGCATCCA GAAGTTTATA
     mp-club64 AATTCTTTAT CAACATTTAT TTTGATTTTT CGGACATCCA GAAGTTTATA
     mz-bell12 GATTCTTTAT CAACATTTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
     mk-bird83 AATTCTCTAT CAACATTTAT TCTGATTTTT TGGACATCCA GAAGTTTATA
     mj-club54 AATTCTTTAT CAACATTTAT TTTGATTTTT CGGACATCCA GAAGTTTATA
    mjk-club44 AATTCTTTAT CAACATTTAT TTTGATTTTT CGGACATCCA GAAGTTTATA
  mp-spindle68 TATTTATAT CAACATTTAT TTTGATTTTT TGGTCATCCA GAAGTTTATA
  mt-spindle38 TATTTATAT CAACATTTAT TTTGATTTTT TGGTCATCCA GAAGTTTATA
  mm-spindle28 TATTTATAT CAACACTTAT TTTGATTTTT TGGACACCCA GAATTTTATA
  mz-spindle18 TATTTATAT CAACACTTAT TTTGATTTTT TGGACACCCA GAAGTTTATA
  mj-spindle58 TATTTATAT CAACACTTAT TTTGATTTTT TGGACACCCA GAAGTTTATA
      mz-bud17 AATTCTGTAT CAACATTTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
      mt-bud37 AGTATTATAC CAACATTTAT TTTGATTCTT TGGTCATCCT GAAGTTTATA

               451                                             500
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Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mj-blister56 TTTTAATTGT TACCGGGA~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mo-blister76 TTTTAATTGT TACCGGGATG GGCCTGGTGG GTTATTTTGA ACCAATTTTT
mt-blister36 TTTTAATTTT ACCGGGATTT ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mjk-bulb45 TTTTAATTTT ACCGGGA~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mj-bulb55 TTTTAATTTT ACCGGGA~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mm-bulb25 TTTTATTTTT ACCGGGAGGA AGTTTAT~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mt-bullet39 TTTTATTTTT TCCGGGGGGG ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mj-bullet59 TTTTAATTTT ACCGGG~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mz-bullet19 TTTTATTTTT TCCCCGGGGG G~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mm-bullet29 TTTTAATTTT ACCGGGGG~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mo-bullet79 TTTTAATTTT TTCCGGGG~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mjk-bullet49 TTTTATTTTT TACCGGGGA~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mp-bullet69 TTTTAATTTT TACCGGGGGG GG~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mj-mice51 TTTTAATTTT TACCGGGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mjk-mice41 TTTTAATTTT TCCCGGGGGA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mt-mice31 TTTTAATTTT TCCGGGGAG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mz-mice11 TTTATTTTTT TCCCCGGGGG A~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mm-mice21 TTTTATTTTT ACCGGGGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mt-bell32 TTTTAATTTT CCCCGGGG~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mm-bell22 TTTTAATTTT GCCCGGGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mjk-bell42 TTTAATTTTT TCCCCGGGGG A~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
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     mt-club34 TTTTAATTTT TTCCGGGGA~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
     mo-club74 TTTTAATTTT ACCGGGGAN~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
     mp-club64 TTTTAATTTT ACCGGGA~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
     mz-bell12 TTTTATTTTT TTCCCGGGGG G~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
     mk-bird83 TTTTAATTTT TCCCCGGGGA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
     mj-club54 TTTTATTTT~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mjk-club44 TTTTAATTTT ACCGGG~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mp-spindle68 TTTTAATTTT TACCGGGGA~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mt-spindle38 TTTTATTT~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mm-spindle28 TTTTAATTTT ACCGGG~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mz-spindle18 TTTTAATTTT ACCGGG~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mj-spindle58 TTTTAATTTT ACCGGG~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
      mz-bud17 TTTTATTTTT TACGGGG~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
      mt-bud37 TTTAATTTTT TCCCGGGGA~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

               501                                  536
  Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
  Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
  Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
  mj-blister56 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
  mo-blister76 CGGTTCTTGG GGAGGGCTAT ATCAGGGGAT ACAACA
  mt-blister36 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
     mjk-bulb45 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
     mj-bulb55 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
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   mm-bulb25 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
 mt-bullet39 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
 mj-bullet59 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
 mz-bullet19 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
 mm-bullet29 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
 mo-bullet79 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mjk-bullet49 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
 mp-bullet69 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mj-mice51 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
  mjk-mice41 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mt-mice31 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mz-mice11 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mm-mice21 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mt-bell32 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mm-bell22 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
  mjk-bell42 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mt-club34 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mo-club74 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mp-club64 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mz-bell12 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mk-bird83 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mj-club54 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
  mjk-club44 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mp-spindle68 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
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mt-spindle38 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mm-spindle28 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mz-spindle18 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mj-spindle58 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
     mz-bud17 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
     mt-bud37 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
```

Appendix 3. 12S Gene Sequence Alignment

Symbol comparison table: pileupdna.cmp CompCheck: 6876

GapWeight: 5
GapLengthWeight: 1

mo-bullet79_pileup_13740.txt MSF: 423 Type: N June 13, 2007 19:36 Check: 2881 ..

Name: mt-bud37 Len: 423 Check: 4335 Weight: 1.00
Name: mz-bud17 Len: 423 Check: 8697 Weight: 1.00
Name: mz-spindle18 Len: 423 Check: 3515 Weight: 1.00
Name: mm-spindle28 Len: 423 Check: 4247 Weight: 1.00
Name: mj-spindle58 Len: 423 Check: 6862 Weight: 1.00
Name: mp-spindle68 Len: 423 Check: 8524 Weight: 1.00
Name: Asphondylia_sphaera_12s Len: 423 Check: 7909 Weight: 1.00
Name: Asphondylia_itoi__12s Len: 423 Check: 932 Weight: 1.00
Name: Asphondylia_gennadii_12s Len: 423 Check: 7522 Weight: 1.00
Name: mm-bulb25 Len: 423 Check: 4012 Weight: 1.00
Name: mj-bulb55 Len: 423 Check: 1299 Weight: 1.00
Name: mz-bulb15 Len: 423 Check: 9622 Weight: 1.00
Name: mjk-mice41 Len: 423 Check: 2153 Weight: 1.00
Name: mt-mice31 Len: 423 Check: 1619 Weight: 1.00
Name: mj-mice51 Len: 423 Check: 4845 Weight: 1.00
Name: mz-mice11 Len: 423 Check: 5135 Weight: 1.00
Name: mm-mice21 Len: 423 Check: 3616 Weight: 1.00

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 Name: mj-club54          Len:   423  Check: 2388  Weight:  1.00
 Name: mz-bell12          Len:   423  Check: 3472  Weight:  1.00
 Name: mt-bell32          Len:   423  Check: 2621  Weight:  1.00
 Name: mjk-bell42         Len:   423  Check: 7095  Weight:  1.00
 Name: mm-bell22          Len:   423  Check: 4975  Weight:  1.00
 Name: mo-club74          Len:   423  Check: 5971  Weight:  1.00
 Name: mt-club34          Len:   423  Check: 7708  Weight:  1.00
 Name: mt-bullet39        Len:   423  Check: 2217  Weight:  1.00
 Name: mj-bullet59        Len:   423  Check: 4263  Weight:  1.00
 Name: mz-bullet19        Len:   423  Check: 3254  Weight:  1.00
 Name: mm-bullet29        Len:   423  Check:  866  Weight:  1.00
 Name: mp-bullet69        Len:   423  Check: 4332  Weight:  1.00
 Name: mjk-bullet49       Len:   423  Check: 9049  Weight:  1.00
 Name: mo-bullet79        Len:   423  Check: 9826  Weight:  1.00

//
                1                                                  50
     mt-bud37 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ CTTAAATTAG
     mz-bud17 ~~~~~~~~~~ ~~~~AGAAAA TAACAAGACT GGGCATATGT ACATATTTTT
  mz-spindle18 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGTGC GGGCATTTGT ACATATTATT
  mm-spindle28 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGTGC GGGCATTTGT .CATATTATT
  mj-spindle58 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGTGC GGGCATTTGT ACATATTATT
  mp-spindle68 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~C GGGCATTTGT ACATATTATT
  Asphondylia_ ~~~tttaaaa ttaattaaaa gcgacgggca atatgtatat attattttaa
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Asphondylia_ ~~~tttaaaa ttaattaaaa gcgacgggca atatgtatat attattttaa
Asphondylia_ ~~~tttaaaa ttaattaaaa gcgacgggcg atatgtatgt attactttta
   mm-bulb25 ~~~~~~~~~~ ~~~~~~~~~~ ~CCGATTGGG AAATGAATAT ATTAAAAATA
   mj-bulb55 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mz-bulb15 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~NNGGGG GGGGCNCGNT GCCGGTNCAG
  mjk-mice41 ~~~~~~~~~~ ~~~~~~~~TG GCATATGAAT ATATTAATAA AATAAATTTA
   mt-mice31 ~~~~~~~~~~ ~~~~~~~~~G GCAGATGAAT ATATTAATAA AATAAATTTA
   mj-mice51 ~~~~~~~~~~ ~~~~~~~~GG GCATGGGCAT ATATTAGTAA AATAAATTTA
   mz-mice11 ~~~~~~~~~~ ~~~~~TGTTG GCATTGGAAT ATATTAATAA AATAAATTTA
   mm-mice21 ~~~~~~~~~~ ~~~~~~~~~~ ~~~TATGAAT ATATTAATAA AATAAATTTA
   mj-club54 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GAAT ATATTTATAA TTTAAATTTA
   mz-bell12 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~AATT ATATTTATAA TTTAAATTTA
   mt-bell32 ~~~~~~~~~~ ~~~~~GGTAG GCATATGGAT ATATTTATAA TTTAAATTTA
  mjk-bell42 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~AT ATATTTATAA TTTAAATTTA
   mm-bell22 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~TGAT ATATTTATAA TTTAAATTTA
   mo-club74 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~CTTTATAA TTTAAATTTA
   mt-club34 ~~~~~~~~~~ ~~~~~~~~~~ ~CATATGAAT ATATTTATAA TTTAAATTTA
 mt-bullet39 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~GTAATTA AATTATCTAA
 mj-bullet59 ~~~~~~~ACC GGGAACTAGG GCAATATGAA TATATTTATA ATATATATTA
 mz-bullet19 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~CAAAT AATCATCTGA
 mm-bullet29 ~~~CATCCAA AAAATTCGGC TTTATTAAAA TATATTAATA AAATATATTA
 mp-bullet69 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~AAAAAAAT TATCATATTA
mjk-bullet49 ~~~~~~~GAC TAAATTTAAG GCATATGAAT ATATTAATAA ATATATATTA
 mo-bullet79 AGGAAAAAAT TCTAAATAGT CAATATGAAT ATATTAATAA ATATATATTA
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               51                                               100
     mt-bud37 CATGTCCATT TAATTATAAA ATTTATTATA ATTTAATTTT AAATACCATT
     mz-bud17 TACATTCATT TAATTATAAA ATTTATTATA ATTTAATTTT AAAT.CCATT
 mz-spindle18 AAAAATTATT CAAATTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
 mm-spindle28 AAAAATTATT CAAATTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
 mj-spindle58 AAAAATTATT CAATTTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
 mp-spindle68 AAAAATTATT CAATTTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
 Asphondylia_ aatattaaaa ttttaaacct ataaaatttt aattttaaat ccattttcat
 Asphondylia_ aatattaaat tattaaattt ataataattt aattttaaat ccatttttat
 Asphondylia_ aatattaaat atttaaatat ataaatattt aattttaaat ccaatttcat
    mm-bulb25 ATATTAAATT TTAACTATAT ATTAAAATTT AATATTACCC TCCAACGTTT
    mj-bulb55 ~~~~~~~~~~ ~~~~~TATAT ATTAAAATTT AATATTA..A ATCCAACTTT
    mz-bulb15 CTTANNGNTC NTNNGGATAT ATTAACAATT TA.ATATTAA ATCCAACTTT
   mjk-mice41 AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
    mt-mice31 AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
    mj-mice51 AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
    mz-mice11 AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
    mm-mice21 AATATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
    mj-club54 AAATAAATAT TTATTTATAA .TTTAATATT AAATCCAATT TAAAAAACAT
    mz-bell12 AAATAAATAT TTATTTATAA .TTTAATATT AAATCCAATT TTAAAAATAT
    mt-bell32 AAAAAAATAT TTA.TTATAA .TTTAATATT AAATCCAATT TAAAAAATAT
   mjk-bell42 AAAAAAATAT TTA.TTATAA .TTTAATATT AAAGCCAATT TAAAAAATAT
    mm-bell22 AAAAAAATAT TTA.TTATAA .TTTAATATT AAAT.CAATT TAAAAAATAT
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mo-club74 AAATAAATAT TTA.TTATAA .TTTAAGATT AAATCCAATT TAAAAAATAT
mt-club34 AAATAAATAT TTA.TTATAA .TTTAATATT AAATCCAATT TAAAAAATAT
mt-bullet39 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATATAACTTA
mj-bullet59 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATATAACTTA
mz-bullet19 AAAAATAATA TATATTAAAA .TTTAATATT AAATCCAACT ACTATAAATT
mm-bullet29 AAAAATAATA TATATTAAAA .TTTAATATT AAATCCAACT ACTATAAATT
mp-bullet69 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATTTTAATTT
mjk-bullet49 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATTTTAATTT
mo-bullet79 AAAAATAATA TATATTATTT .TTTAATATT AAATTCAATT ATTTTAATTT

101 150
mt-bud37 TTCAAAAATT TATTACAAAA ATTTATTTAA AATTTATATT AATGTATCTC
mz-bud17 TTCAAAAATT TATTACAAAA ATTTATTTAA AATTTATATT AATGTATCTC
mz-spindle18 TCATAA.TTT TTTTACAAAA ATTAATTCAA AAACATTTTT ATTGTATTTC
mm-spindle28 TCATAA.TTT TTTTACAAAA ATTAATTCAA AAATATTTTT ATTGTATTTC
mj-spindle58 TCATAA.TTT TTTTACAAAA TTTAATTCAA AAATATTTTT ATTGTATTTC
mp-spindle68 TCATAATTTT TTTTACAAAA TTTAATTCAA AAATATTTTT ATTGTATTTC
Asphondylia_ ttaaatatta aaatttaaaa ttcaatttta aaatttattt aatgtatttc
Asphondylia_ ttaaatatta aaatttaaaa tccaatt... aaatttattt aatgtatttc
Asphondylia_ ttaaatatta aaacttaaaa tcc.atatat aaataaattt aatgtatttc
mm-bulb25 ATTAATATAT AACAATAAAA ATTCTGCAAA AATTAAAATA AATGTATTTC
mj-bulb55 ATTAATATAT AACAATAAAA ATTCTGCAAA AATTAAAATA AATGTATTTC
mz-bulb15 ATTAGTATAT AACAATAAAA ATTCTGCAGA ACTTAAAATA AATGTATTTC
mjk-mice41 ATT....TAC AATAATAATG CTATAT.AAA A..TAATATT AATGTATTTC

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    mt-mice31 ATT....TAC AATAATAATG CTATAT.AAA A..TAATGTT AATGTATTTC
    mj-mice51 ATT....TAC AATAATAATG CTATAT.AAA A..TAGTATT AATGTATTTC
    mz-mice11 ATT....TAC AATAATAATG CTGTAT.AAA A..TAATATT AATGTAGTTC
    mm-mice21 ATT....TAC AATAATAATG CTATATCCAA A..TAAGAAT AATGTATTTC
    mj-club54 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AATGTATTTC
    mz-bell12 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AAGGTATTTC
    mt-bell32 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AATGTATTTC
   mjk-bell42 TTTACAATAT AA.AATCCAT AGAAATTATA A..AATTATT AATGTATTTC
    mm-bell22 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AATGTATTTC
    mo-club74 CTTACAATAT AAGAAGCCAT ACGAATTATA A..AATTATT AATGTATTTC
    mt-club34 CTTACAATAT AA.AATCCAT AAGACTTATA A..AATTATT AAGGTATTTC
  mt-bullet39 TATTACAATA AATAATAATA TAATACCATT A..TAAATCA AATGTATTTA
  mj-bullet59 TATTACAATA AATATAATAA TTA....ATT A..TAAATCA AATGTATTTC
  mz-bullet19 TATTACAATA AATACTAAAT .......AAA A..TAAATTT AATGTATTTC
  mm-bullet29 TATTACAATA AATACTAAAT .......AAA A..TAAATTT AATGTATTTC
  mp-bullet69 ATTACAATAA ATTATTAAAT ATA...AAAT A..TAAATTA AATGTATTTC
 mjk-bullet49 ATTACAATAA ATTATTAAAT ATA...AAAT A..TAAATTA AATGTATTTC
  mo-bullet79 ATTACAATAA ATTAATAAAT ATA...AAAT A..TAAATTA AATGTATTTC

              151                                              200
     mt-bud37 A.TTTAAAAT TTAAATATAA AATGAATTAT GATTTGACAT TTTATATT.A
     mz-bud17 A.TTTAAAAT TTAAATATAA AATGAATTAT GATTTGACAT TTTATATT.A
 mz-spindle18 A.TTTTATTT TTATATATTT AATGAATTTT GATTTGACTT TAAATTTAAA
 mm-spindle28 A.TTTTATTT TTATATATTT AATGAATTTT GATTTGACTT TAAATTTAAA
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mj-spindle58 A.TTTTATTT TTATATATTT AATGAATTTT GATTTGACTT CAAATTTAAA
mp-spindle68 A.TTTTAATT TTATATATTT AATGAATTTT GATTTGACTT CTAATCTTAA
Asphondylia_ atttt.aatc ttatactttt aatatattat gatttgaaat aaattaat..
Asphondylia_ attttaaacc ttaaattttt aatatattat gatttgaatt aaattata..
Asphondylia_ atttttaatc ttttatttta aatatattat gatttgaatt aaattaat..
    mm-bulb25 A.TTTAAAAT TTAATTATTA AATATATTAT GATTTGAATT TTGTTATAT.
    mj-bulb55 A.TTTAAAAT TTAATTATTA AATATATTAT GATTTGAATT TTAATATAT.
    mz-bulb15 A.TTTAAAAT TTAATTATTA AATATATTAT GATTTGAATT TTACTATATA
  mjk-mice41 A.TTTAAATC TTAAATATGA AATATATTAT GATTTGAAAT TTA..TTAT.
    mt-mice31 A.TTTAAATC TTAAATATGA AATATATTAT GATTTGAAAT TTA..TTAT.
    mj-mice51 A.TTTAAATC TTAAATATGA AATATATTAT GATTTGAAAT TTA..TTAT.
    mz-mice11 A.TTTAAATC TTAAATATGA AATATATTAT GATGTGAAGT TTA..TTAT.
    mm-mice21 A.TTTAAATC TTAAATATAA AATATATTAT GATTTGAAAT TTATTTTTT.
    mj-club54 A.TTTAAATT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
    mz-bell12 A.TTTAAATT TTAAATATAA AATATATTAG GATTGGAAAT TATTTTAAT.
    mt-bell32 A.TTTAAAAT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
  mjk-bell42 A.TTTAAAAT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
    mm-bell22 A.TTTAAAAT TTAAATATAA AAGATATTAT GATTTGAAAT TATTTTAAT.
    mo-club74 A.TTTAAACT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
    mt-club34 A.TTTAAACT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
  mt-bullet39 A.TTTAAATC TTAAATATAA AATATATTAT AATTTGAAAT TTA.T.TAA.
  mj-bullet59 A.TTTAAATC TTAAATATAA AATATATTAT AATTTGAAAT TTATT.TTA.
  mz-bullet19 A.TCTAAATC TTAAATATAA AATATATTAT GATTTGAATT TTTTTAATA.
  mm-bullet29 A.TCTAAATC TTAAATATAA AATATATTAT GATTTGAATT TTTTTAAT..
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   mp-bullet69 A.TTTAAACC TTAAATATAA AATATATTAT GATTTGAAAT TAATTACTA.
  mjk-bullet49 A.TTTAAACC TTAAATATAA AATATATTAT GATTTGAAAT TAATTACTA.
   mo-bullet79 A.TTTAAACC TTAAATATAT AATATATTAT GATTTGAAAT TAATTACTA.

               201                                                250
      mt-bud37 TTCAAATATT TAAATAATAA ATTTTTTAAA AATTATTTTA TGACAACAAT
      mz-bud17 TTCAAATATT TAAATAATAA ATTTTTTAAA AATTATTTTA TGACAACAAT
  mz-spindle18 ATTAAATTTT TCAATAATAA A..TCCAAAA AATTATTTTA AGACAACAAT
  mm-spindle28 ATTAAATTTT TCAATAATAA A..TCCAAAA AATTATTTTA AGACAACAAT
  mj-spindle58 ATTAAATTTT TCAATAATAA A..TCCAAAA AATTATTTTA AGACAACAAT
  mp-spindle68 ATTAAATTTT TTAATAATAA A..TTTAAAA AATTATTTTA AGACAACAAT
  Asphondylia_ .aaaaataaa atttattaat tttttattac aaaattaatt aaacaacaat
  Asphondylia_ .ataaaataa ttattaataa attttattat aaaattaatt aaacaacaat
  Asphondylia_ ....aatata atatttatta atattttact aaaatcaatt aaacaacaat
     mm-bulb25 ..AAAAAATA AAATTAATTT TTTATAAAAT AAAATTAATT AAACAACAAT
     mj-bulb55 .AAAAAAATA AAATTAATTT TTTATAAAAT AAAATTAATT AAACAACAAT
     mz-bulb15 AAAANAAANA AANTAATTTT TTNAAAAANA AAANTNATTN AACCNCCCNT
    mjk-mice41 .AAAAAAANA NTANTAANAN TTTTTT...N AAANTNATTN AACCACCATN
     mt-mice31 .AAAAAAAAA ATTATAAAAA TTTTTT...T AAAATTANTT AACCACCANT
     mj-mice51 .AAAAAAAAT ATTATTAATA TTTTTT...T AAAATTAATT AAACAACAAT
     mz-mice11 ..AAAAAAAT ACTATTAATA TTTTTT...T AAAATTAATT AAACAACAAT
     mm-mice21 .AAAAAAATA TTATTAATAT TTTTTT...T AAAATTAATT AAACAACAAT
     mj-club54 .AAAAATTTT TAATTAATAT TTT.TTTATT AAAATTAATT AAACAACAAT
     mz-bell12 .AAAAATTTT TAATTAATA. TTT.TTTATT AAAATTAATA AAACAACAAT
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    mt-bell32 .AAAAATATT TAATTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
   mjk-bell42 .AAAAATATT TAAGTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
    mm-bell22 .AAAAATATT TAATTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
    mo-club74 .AAAAAATTT TAATTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
    mt-club34 .AAAAATTTT TAATTAATA. ..T.TTTATT AAAATTAATT AACCAACAAT
  mt-bullet39 .AAAAAAATA AAATTAATTT TTTATT.AAT AAAATCAATT AAACAACAAT
  mj-bullet59 .AAAAAAATA AAATTAATTT TTTATT.AAT AAAATCAATT AAACAACAAT
  mz-bullet19 .AAAAAAATA AAATTAATTT TTTATTAAAT AAAATTAATT AAACAACAAT
  mm-bullet29 .AAAAAAATA AAATTAATTT TTTATTAAAT AAAATTAATT AAACAACAAT
  mp-bullet69 .ATAAAAATA AAATTAATTT TCTATTTAAT AAAATCAATT AAACAACAAT
 mjk-bullet49 .ATAAAAATA AAATTAATTT TCTATTTAAT AAAATCAATT AAACAACAAT
  mo-bullet79 .ACAAAAATA AAATTAATTT TCTATTTAAT AAAATCAATT AAACAACAAT

              251                                            300
     mt-bud37 ATACAATTTA ATT.AATTTA AATATATTTT ATTGTGTATT ATC.AATTAA
     mz-bud17 ATACAATTTA ATT.AATTTA AATATATTTT ATTGTGTATT ATC.AATTAA
 mz-spindle18 ATACAATTTA ATT.AATATA AATAAATTAT AATGTGGATT ATC.AATTAA
 mm-spindle28 ATACAATTTA ATT.AATATA AATAAATTAT AATGTGGATT ATC.AATTAA
 mj-spindle58 ATACAATTTA ATT.AATATA AATAAATTAT AATGTGGATT ATC.AATTAA
 mp-spindle68 ATACAATTTA ATT.AATATA AATAAATTAT AAGGTGGATT ATC.AATTAA
 Asphondylia_ atataattta a.ttaatata agtttattta aatgtgtatt atcaaattaa
 Asphondylia_ atataatttt a.ttaaatta agtatatttt aatgtgtatt atc.aattaa
 Asphondylia_ atataatttt atttaataaa agtatattat aatgtgtatt atc.aattaa
    mm-bulb25 ATATAATTTT ATTAAA.TTA AATATATTAA ATTGGGGATT ATC.AATTAA
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  mj-bulb55 ATATAATTTT ATTAAA.TTA AATATATTAA ATTGGGGATT ATC.AATTAA
  mz-bulb15 NNNAANTTTT ATNANA.TTA ANANTATAAA NTGGGGTATN ACC.ANTNAA
 mjk-mice41 NNTNANCCTN ANAAANTTNA AAAANNTAAA NTGGGGANTA ACC.ANTNAN
  mt-mice31 NTNTAANCTN AANAAATTNA AANANNTNAN ATGGGGAATN ANC.AATNAA
  mj-mice51 ATATAATCTT AATAAATTTA AATATATTGT ATTGTGTATT ATC.AATTAA
  mz-mice11 ATATAATCTC AATAAATTTA AATATATTAT ATTGTGTGTG ATC.AATTAA
  mm-mice21 ATATAATTTT AATAAATTTA AATATATTAT ATTGGGGATT ATC.AATTAA
  mj-club54 ATATAATTTT AATAAA.TTA AATATATTAT ATTGTGTATT ATC.AATTAA
  mz-bell12 ATATAATTTT AATAAA.TTA AATATATTAT GTTGTGTATT ATC.AATTAA
  mt-bell32 ATATAATTTT AATAAA.TCA AACATATTAT ATTGTGTATT ATC.AATTAA
 mjk-bell42 ATATAATTTT AATAAA.TCA AACATATTAT ATTGTGTATT ATC.AATTAA
  mm-bell22 ATATAATTTT AATAAA.TCA AACATATTAT ATTGTGTATT ATC.AATTAA
  mo-club74 ATATAATTTT AATAAA.TTA AATATATTAT ATTGTGTATT ATC.AATTAA
  mt-club34 ATATAATTTT AATAAA.TTA AATATATTAT ATGGGGTATT ATC.AATTAA
mt-bullet39 ATATAATTTT A.TAAA.ATA AATATATTAT AACGTGTATT ATC.TATTAA
mj-bullet59 ATATAATTTT A.TAAA.ATA AATATATTAT AACGTGTATT ATC.TATTAA
mz-bullet19 ATATAATTTA A.TAAA.ATA AATATATTAT AATGTGTATT ATC.AATTAA
mm-bullet29 ATATAATTTA A.TAAA.ATA AATATATTAT AATGTGTATT ATC.AATTAA
mp-bullet69 ATATAATTTA ATTAAA.ATA AGTATATTAT AATGTGTATT ATC.AATTAA
mjk-bullet49 ATATAATTTA ATTAAA.ATA AGTATATTAT AATGTGTATT ATC.AATTAA
mo-bullet79 ATATAATTTA ATTAAA.ATA AGTATATTAT AATGTGTATT ATC.AATTAA

            301                                              350
    mt-bud37 TTAACAAGAT CCTCTAATTT TAAAAAATAC TGCCAATTTA TTTAATTTTT
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mz-bud17	TTAACAAGAT	CCTCTAATTT	TAAAAAATAC	TGCCAATTTA	TTTAATTTTT
mz-spindle18	TAAACAAAAT	CCTTTAATTT	TTAAAAATAC	TGCCATTTTA	TTTAATTTTA
mm-spindle28	TAAACAAAAT	CCTTTAATTT	TTAAAAATAC	TGCCATTTTA	TTTAATTTTA
mj-spindle58	TAAACAAAAT	CCTCTAATTT	TTAAAAATAC	TGCCACTTTA	TTTAATTTTA
mp-spindle68	TAAACAAAAT	CCTTTAATTT	TTAAAAATAC	TGCCATTTTA	TTTAATTTTT
Asphondylia_	ttaacaaaat	cctctaattt	taaataatac	taccaaatta	tttaatttt.
Asphondylia_	taaacaaaat	cctctaattt	taaataatac	taccaaatta	tttaatttt.
Asphondylia_	taaacaaaat	cctctaattt	taaataatac	caccaaatta	tttaattttc
mm-bulb25	TTAACAAAAT	CCCCTAATTT	TTAAAAA.TA	CTACCAAATT	AATTAATTTT
mj-bulb55	TTAACAAAAT	CCTCAAATTT	TTAAAAA.TA	CTACCAAATT	AATTAATTTT
mz-bulb15	TNACCAAAAC	CCCCNANTTT	TAAAAANGNN	CCNCNAAATN	AATTANTTTC
mjk-mice41	TNACCAAANC	CCCCNANTTT	TNAAAANNCC	NCCCAANTNA	NTNANTTTTN
mt-mice31	TTANCAAANN	CCNCTAATTT	TNAAAAANNC	NNCCAAATNA	ATNANTTTTN
mj-mice51	TTAACAAAAT	CCTCTAATTT	TTAAGAATAC	TACCAAATTA	ATTAAGTTTT
mz-mice11	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	ATTAATTTTT
mm-mice21	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	ATTAATTTTT
mj-club54	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mz-bell12	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mt-bell32	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mjk-bell42	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mm-bell22	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAGATTA	TTTAATTTTA
mo-club74	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mt-club34	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mt-bullet39	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA

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  mj-bullet59 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTA
  mz-bullet19 TTGACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTT
  mm-bullet29 TTGACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTT
  mp-bullet69 TAAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA ATTAATTTTT
 mjk-bullet49 TCAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA CTTAATTTTT
  mo-bullet79 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTT

              351                                                400
     mt-bud37 TTTAAATTAA AAATTAATTT TTTTAA...A AAAAAAAAGG GGATCTAATC
     mz-bud17 TTTAAATTAA AAATTAATTT TTTTAA...A AAAAAAAAGG GTATCTAAAC
  mz-spindle18 TTATAAT.TT TTACTAATTA TTTTTA...A TCTTAAAATT AAAATAATAG
  mm-spindle28 TTATAAT.TT TTACTAATTA TTTTTA...A TCTTAAAATT AAAATAATAG
  mj-spindle58 ATCAATC.TA CTACTAATTA TTTATT...A TCATTAAAAT AATAGGGTAG
  mp-spindle68 ATATATTATT TTACTAATTA TTTATT...A AAATTAAAAT AATAGGGTAT
  Asphondylia_ ..taaaaata aaaatttact aattaaaaa. aaaattaatc cttataata~
  Asphondylia_ ..ttttaatt taataataat tattaaaaat ttaataaatt tatataata~
  Asphondylia_ aaaaaaaatt taatattact tattaaaata ttattaaact aatataata~
     mm-bulb25 CTCTTTTTAT AGA.ATAATA GGCCAA..TA AAT.TATATT ATTATAATAG
     mj-bulb55 CTCTTTTAAT AGA.ATAATA ATTAAT..AA ACTCTATATT ATTATAATAG
     mz-bulb15 CNCTTTTAAN AAA.ATANNA ATTAA....N AANTCNTATT ATNANAGNGG
    mjk-mice41 AAAANANTTT TNA.ANANTN ANAN.....C CCTNAAAAAA TTNNNANAGG
     mt-mice31 AANAANATTT TNA.AAAATT AANA.....N CCNTAAAAAA NTTATANNNG
     mj-mice51 AATAATATTT TTA.ATAATT AATA.....T CCATAAAAAA ATTATAATAG
     mz-mice11 AATAATATTT TTA.ATAATT AATA.....T CCATAAAAAA ATTATAATAG
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    mm-mice21 AATATCATAT TTA.ATAATT AGTA.....T TCATAAAAAA ATTATAATAG
    mj-club54 AAATAAAAAA TTA.ATAATT AAATAT...A TAATAAAAAA ATTATAATAG
    mz-bell12 AAATAAAAAA TTA.ATAATT AAATATATAA TAATAAAAAA ATTAGAATAG
    mt-bell32 AAATAAAAAA ATT.AATAAT TAAATT...T ATATATAAAA ATTATAATAG
   mjk-bell42 AAATAAAAAA ATT.AATAAT TAAATT...T ATATATAAAA ATTATAATAG
    mm-bell22 AAATAAAAAA ATT.AATAAT TAAATT...T ATATATAAAA ATTATAATAG
    mo-club74 AAATAAAAAT TTA.ATAATT AAATAT...A TAATAAAAAA ATTATAATAG
    mt-club34 AAATAAAAAT TTA.ATAATT AAATAT...A TAATAAAAAA ATTATAATAG
  mt-bullet39 ATTTTTTAAA TTA.ATAATT AATA.....C AAATAAAAAA ATTATAATAG
  mj-bullet59 ATTTTTTAAA TTA.ATAATT AATA...... GAATAAAAAA ATTATAATAG
  mz-bullet19 A.TTTTAATA TTA.ATAATT AATA.....A AA..AAAAAT ATTATAATAG
  mm-bullet29 A.TTTTAATA TTA.ATAATT AATA.....A AA..AAAAAT ATTATAATAG
  mp-bullet69 A.TTTTAAAA TTA.ATAATT AATA.....T AATTAAAAAA ATTATAATAG
 mjk-bullet49 A.CTTTAAAA TTA.ATAATT AATA.....T AATTAAAAAA ATTATAATAG
  mo-bullet79 A.TTTTAAAA TTA.ATAATT AATA.....T AATTAAAAAA ATTATAATAG

              401                     423
     mt-bud37 CTAGTTTAGT CA~~~~~~~~ ~~~
     mz-bud17 CTAATTTA~~ ~~~~~~~~~~ ~~~
 mz-spindle18 GGTATCTAAT CCCTAGTTTA ~~~
 mm-spindle28 GGTATCTAAT CCTAGTTTA~ ~~~
 mj-spindle58 GGAATCCTAG CCTA~~~~~~ ~~~
 mp-spindle68 CTAATCCTAG TTTA~~~~~~ ~~~
  Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~
```

```
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~
   mm-bulb25 GGCATCTAAT CCTAGTTTA~ ~~~
   mj-bulb55 GGGATCTAAT CCTAGTTTAA ~~~
   mz-bulb15 GGNACCNANC CCNNNNTAAN ~~~
  mjk-mice41 GGANCCNANC CCAGGTTAA~ ~~~
   mt-mice31 GGNANCTAAC CCNNGTTAA~ ~~~
   mj-mice51 GGGATCTAAT CCTAGTTTA~ ~~~
   mz-mice11 GGGGGCTAAT CCTAGTTTAT ATA
   mm-mice21 GGGATCTAAT CCTAGTTTA~ ~~~
   mj-club54 GGTATCTAAT CCTAGTTTA~ ~~~
   mz-bel112 GGTATCTAAT CCAAGTTTA~ ~~~
   mt-bel132 GGTATCTAAT CCTAGTTTA~ ~~~
  mjk-bel142 GGTATCTAAT CCTAGTTTA~ ~~~
   mm-bel122 GGTATCTAAT CCTAGTTTA~ ~~~
   mo-club74 GGTATCTAAT CCTAGTTTA~ ~~~
   mt-club34 GGTATCTAAC CCTAGTTTA~ ~~~
 mt-bullet39 GGTATCTAAT CCTAGTTTAA ~~~
 mj-bullet59 GGTATCTAAT CCTAGTTTAA ~~~
 mz-bullet19 GGTATCTAAT CCTAGTTTAA ~~~
 mm-bullet29 GGTATCTAAT CCTAGTTTAA ~~~
 mp-bullet69 GGTATCTAAT CCAAGTTTAA ~~~
mjk-bullet49 GGTATCTAAT CCTAGTTTAA ~~~
 mo-bullet79 GGTATCTAAT CCTAGTTTAA ~~~
```

Appendix 4. COI Gene Genetic Distances

Genetic Distances

Calculated over: 44 to 440
Considering all base positions
Correction method: Kimura 2-parameter
Distances are: estimated number of substitutions per 100 bases
Symmatrix version 1
Number of matrices: 1

//
Matrix 1, dimension: 38

Key for column and row indices:

1 Asphondylia_sphaera-Asphodyliini
2 Asphondylia_gennadii-Asphodyliini
3 Asphondylia_itoi-Asphodyliini
4 mj-blister56
5 mo-blister76
6 mt-blister36
7 mjk-bulb45
8 mj-bulb55
9 mm-bulb25
10 mt-bullet39

11 mj-bullet59
12 mz-bullet19
13 mm-bullet29
14 mo-bullet79
15 mjk-bullet49
16 mp-bullet69
17 mj-mice51
18 mjk-mice41
19 mt-mice31
20 mz-mice11
21 mm-mice21
22 mt-bell32
23 mm-bell22
24 mjk-bell42
25 mt-club34
26 mo-club74
27 mp-club64
28 mz-bell12
29 mk-bird83
30 mj-club54
31 mjk-club44
32 mp-spindle68
33 mt-spindle38
34 mm-spindle28

35 mz-spindle18
36 mj-spindle58
37 mz-bud17
38 mt-bud37

Matrix 1: Part 1

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.00	12.94	16.02	31.94	35.96	34.82	25.29	25.64	29.73	25.64	26.73	23.90
2		0.00	14.13	28.91	32.33	33.62	23.54	23.20	28.19	23.89	27.08	23.54
3			0.00	30.79	33.90	31.29	24.60	25.65	26.41	23.90	23.88	24.23
4				0.00	22.50	19.51	23.81	22.10	27.01	22.86	27.08	24.93
5					0.00	27.95	25.57	25.22	27.72	29.66	32.33	28.55
6						0.00	27.08	26.73	30.44	30.52	30.20	29.07
7							0.00	2.83	9.43	18.53	20.16	16.62
8								0.00	9.73	19.50	20.83	17.88
9									0.00	24.26	24.58	20.85
10										0.00	6.60	11.44
11											0.00	14.44
12												0.00
13												
14												
15												
16												
17												
18												
19												
20												

l 21 l

l 22 l

l 23 l

l 24 l

l 25 l

l 26 l

l 27 l

l 28 l

l 29 l

l 30 l

l 31 l

l 32 l

l 33 l

l 34 l

l 35 l

l 36 l

l 37 l

l 38 l

Matrix 1: Part 2

	13	14	15	16	17	18	19	20	21	22	23	24
1	23.20	21.51	21.50	20.83	22.22	22.22	22.22	22.57	20.51	21.49	21.49	21.49
2	22.85	20.49	21.16	21.16	19.18	19.18	19.18	19.51	19.50	19.50	19.50	19.50
3	23.54	21.16	21.16	21.16	22.51	22.51	22.51	22.85	20.49	21.17	21.17	21.17
4	24.58	22.52	22.51	23.20	23.88	23.88	23.88	23.88	22.51	20.49	20.49	20.49
5	29.31	28.17	27.81	28.17	27.08	27.08	27.08	27.08	27.82	28.17	28.17	28.17
6	28.31	26.09	26.10	26.09	27.17	27.17	27.17	27.17	26.80	24.66	24.66	24.66
7	16.62	15.06	15.68	15.36	17.24	17.24	17.24	17.24	16.93	14.13	14.13	14.13
8	17.88	15.99	16.61	16.30	17.24	17.24	17.24	17.24	17.24	15.36	15.36	15.36
9	20.85	18.20	18.85	18.52	20.84	20.84	20.84	20.84	20.50	18.86	18.86	18.86
10	11.15	11.73	11.44	11.44	12.03	12.03	12.03	12.33	11.15	10.27	10.27	10.27
11	14.13	14.13	13.83	13.83	13.24	13.24	13.24	13.54	12.63	12.03	12.03	12.03
12	0.76	6.65	6.36	6.08	11.82	11.82	11.82	12.12	10.90	11.17	11.17	11.17
13	0.00	6.08	5.80	5.53	11.82	11.82	11.82	12.12	10.90	10.87	10.87	10.87
14		0.00	0.76	0.76	9.13	9.13	9.13	9.42	8.84	8.27	8.27	8.27
15			0.00	0.51	9.71	9.71	9.71	10.00	9.41	9.12	9.12	9.12
16				0.00	9.42	9.42	9.42	9.71	9.13	8.55	8.55	8.55
17					0.00	-0.00	-0.00	0.25	2.58	6.60	6.60	6.60
18						0.00	-0.00	0.25	2.58	6.60	6.60	6.60
19							0.00	0.25	2.58	6.60	6.60	6.60
20								0.00	2.84	6.88	6.88	6.88

21	0.00	5.23	5.23	5.23
22		0.00	-0.00	-0.00
23			0.00	-0.00
24				0.00
25				
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				
37				
38				

Matrix 1: Part 3

	25	26	27	28	29	30	31	32	33	34	35	36
1	20.16	20.16	20.49	20.82	20.82	19.83	20.82	23.62	23.62	25.37	25.72	26.37
2	18.21	18.21	18.53	18.85	18.85	18.53	18.85	22.57	22.57	23.61	23.96	24.23
3	20.50	20.50	20.84	20.84	21.16	20.84	21.16	23.96	23.96	23.61	23.96	24.23
4	20.49	20.49	20.16	20.82	20.16	20.49	19.45	21.26	20.91	24.67	25.02	23.91
5	29.66	29.66	28.91	29.66	28.55	28.54	26.99	26.15	26.15	24.66	25.02	24.23
6	25.01	25.01	25.02	24.67	25.02	25.02	25.65	28.04	27.67	31.43	31.79	29.05
7	15.36	15.36	15.36	15.67	15.05	15.36	15.32	20.91	20.91	23.62	23.98	23.55
8	16.61	16.61	16.61	16.93	16.30	16.61	16.56	21.24	21.24	23.96	24.32	23.89
9	20.52	20.52	20.17	20.51	20.51	20.84	20.79	25.36	25.36	25.72	26.08	25.29
10	10.56	10.56	10.56	9.98	9.40	10.56	11.15	22.96	22.63	22.95	23.31	22.88
11	12.33	12.33	12.33	11.73	11.44	12.33	12.63	24.66	24.31	25.72	26.08	24.93
12	10.57	10.57	10.87	11.17	9.70	10.87	11.46	21.23	20.90	23.30	23.66	22.18
13	10.28	10.28	10.57	10.87	9.41	10.57	11.16	20.57	20.24	22.96	23.32	21.85
14	7.99	7.99	8.84	8.55	8.27	8.84	9.41	20.89	20.56	22.60	22.96	20.85
15	8.55	8.55	9.41	9.12	8.84	9.41	9.98	20.23	19.90	21.59	21.95	19.87
16	7.99	7.99	8.84	8.55	8.27	8.84	9.41	19.91	19.58	22.27	22.63	20.53
17	7.17	7.17	7.17	6.89	6.89	7.17	7.73	21.22	20.89	23.61	23.96	21.49
18	7.17	7.17	7.17	6.89	6.89	7.17	7.73	21.22	20.89	23.61	23.96	21.49
19	7.17	7.17	7.17	6.89	6.89	7.17	7.73	21.22	20.89	23.61	23.96	21.49
20	7.45	7.45	7.45	7.17	7.18	7.45	8.01	21.56	21.22	23.96	24.31	21.83

21	5.78	5.78	5.78	5.50	5.51	5.78	6.33	19.24	18.92	22.92	23.27	20.82
22	3.10	3.10	3.10	2.31	3.10	3.10	3.62	18.26	17.94	21.56	21.89	20.16
23	3.10	3.10	3.10	2.31	3.10	3.10	3.62	18.26	17.94	21.56	21.89	20.16
24	3.10	3.10	3.10	2.31	3.10	3.10	3.62	18.26	17.94	21.56	21.89	20.16
25	0.00	-0.00	1.79	2.58	1.80	1.79	2.57	19.57	19.24	22.23	22.58	21.83
26		0.00	1.79	2.58	1.80	1.79	2.57	19.57	19.24	22.23	22.58	21.83
27			0.00	1.80	2.06	0.51	1.53	19.57	19.24	22.23	22.58	21.49
28				0.00	2.32	1.80	2.84	19.89	19.57	22.57	22.92	21.16
29					0.00	2.06	3.10	19.24	18.92	20.89	21.23	20.16
30						0.00	1.02	19.89	19.57	21.90	22.24	21.16
31							0.00	19.89	19.56	21.89	22.24	21.16
32								0.00	0.25	11.86	11.84	9.78
33									0.00	11.55	11.54	9.48
34										0.00	0.51	2.05
35											0.00	2.05
36												0.00
37												
38												

Matrix 1: Part 4

	37	38
1	22.17	26.11
2	21.84	25.40
3	22.17	27.58
4	23.20	23.60
5	25.36	29.38
6	33.24	27.60
7	23.57	23.55
8	25.32	24.95
9	26.72	27.51
10	21.56	26.80
11	21.85	30.51
12	22.87	25.74
13	22.54	25.37
14	21.84	22.58
15	21.17	22.94
16	21.84	22.58
17	21.83	25.73
18	21.83	25.73
19	21.83	25.73
20	22.17	26.09

21		21.16	24.66
22		22.21	22.23
23		22.21	22.23
24		22.21	22.23
25		20.85	22.92
26		20.85	22.92
27		21.20	23.26
28		21.53	22.92
29		20.22	23.61
30		21.53	22.57
31		21.17	22.85
32		19.23	25.47
33		19.23	25.47
34		20.55	26.52
35		20.55	26.16
36		21.16	26.08
37		0.00	26.54
38			0.00

#NEXUS

```
begin trees;
utree Tree_1 = (((((((((((('Asphondylia_sphaera-Asphodyliini':7.30,'Asphondylia_gennadii-Asphodyliin'
:5.64):0.79,'Asphondylia_itoi-Asphodyliini':7.82):4.71,(((('mj-blister56'
:7.60,'mt-blister36':11.91):1.12,'mo-blister76':14.35):2.20,
'mt-bud37':12.68):1.31,((('mp-spindle68':0.25,'mt-spindle38'
:0.00):4.34,(('mm-spindle28':0.11,'mz-spindle18':0.40):1.33,
'mj-spindle58':0.46):5.17):4.45,'mz-bud17':10.26):1.13):1.05)
:1.61,(('mjk-bulb45':1.07,'mj-bulb55':1.76):1.53,'mm-bulb25'
:6.64):6.27):2.36,(('mz-bullet19':0.52,'mm-bullet29':0.24)
:3.71,('mo-bullet79':0.23,('mjk-bullet49':0.45,'mp-bullet69'
:0.06):0.27):1.68):2.17):0.56,('mt-bullet39':2.43,'mj-bullet59'
:4.17):4.13):1.11,(((('mj-mice51':-0.01,'mz-mice11':0.26)
:0.01,'mjk-mice41':-0.01):0.00,'mt-mice31':-0.00):1.90,'mm-mice21'
:0.68):2.31):1.11,(('mt-bell32':0.00,'mm-bell22':-0.00):0.00,
'mjk-bell42':0.00):1.34):0.32,'mz-bell12':0.93):0.31,(('mt-club34'
:0.00,'mo-club74':-0.00):0.88,'mk-bird83':0.92):0.21):0.27,
('mp-club64':0.30,('mj-club54':0.07,'mjk-club44':0.95):0.21)
:0.27):0.00;
endblock;
```

```
Symbol comparison table: pileupdna.cmp  CompCheck: 6876

                   GapWeight: 5
             GapLengthWeight: 1

PileUp  MSF: 536  Type: N  June 7, 2007 17:01  Check: 1087 ..

 Name: Asphondylia_sphaera-Asphodyliini  Len:   536  Check:  637  Weight:  1.00
 Name: Asphondylia_gennadii-Asphodyliini  Len:   536  Check:  911  Weight:  1.00
 Name: Asphondylia_itoi-Asphodyliini  Len:   536  Check: 5604  Weight:  1.00
 Name: mj-blister56     Len:   536  Check: 6062  Weight:  1.00
 Name: mo-blister76     Len:   536  Check: 2456  Weight:  1.00
 Name: mt-blister36     Len:   536  Check: 3362  Weight:  1.00
 Name: mjk-bulb45       Len:   536  Check: 4371  Weight:  1.00
 Name: mj-bulb55        Len:   536  Check: 2505  Weight:  1.00
 Name: mm-bulb25        Len:   536  Check: 5539  Weight:  1.00
 Name: mt-bullet39      Len:   536  Check: 4124  Weight:  1.00
 Name: mj-bullet59      Len:   536  Check: 4340  Weight:  1.00
 Name: mz-bullet19      Len:   536  Check: 7475  Weight:  1.00
 Name: mm-bullet29      Len:   536  Check: 4713  Weight:  1.00
 Name: mo-bullet79      Len:   536  Check: 4503  Weight:  1.00
 Name: mjk-bullet49     Len:   536  Check: 7592  Weight:  1.00
 Name: mp-bullet69      Len:   536  Check: 7579  Weight:  1.00
 Name: mj-mice51        Len:   536  Check: 7909  Weight:  1.00
```

```
Name: mjk-mice41       Len:   536  Check: 1996  Weight:  1.00
Name: mt-mice31        Len:   536  Check: 6747  Weight:  1.00
Name: mz-mice11        Len:   536  Check: 2413  Weight:  1.00
Name: mm-mice21        Len:   536  Check: 8856  Weight:  1.00
Name: mt-bell32        Len:   536  Check: 5561  Weight:  1.00
Name: mm-bell22        Len:   536  Check: 5775  Weight:  1.00
Name: mjk-bell42       Len:   536  Check: 4717  Weight:  1.00
Name: mt-club34        Len:   536  Check: 4772  Weight:  1.00
Name: mo-club74        Len:   536  Check: 4233  Weight:  1.00
Name: mp-club64        Len:   536  Check: 7827  Weight:  1.00
Name: mz-bell12        Len:   536  Check: 7820  Weight:  1.00
Name: mk-bird83        Len:   536  Check: 7944  Weight:  1.00
Name: mj-club54        Len:   536  Check: 7085  Weight:  1.00
Name: mjk-club44       Len:   536  Check: 4477  Weight:  1.00
Name: mp-spindle68     Len:   536  Check: 1892  Weight:  1.00
Name: mt-spindle38     Len:   536  Check: 7329  Weight:  1.00
Name: mm-spindle28     Len:   536  Check: 7015  Weight:  1.00
Name: mz-spindle18     Len:   536  Check: 3732  Weight:  1.00
Name: mj-spindle58     Len:   536  Check: 2869  Weight:  1.00
Name: mz-bud17         Len:   536  Check: 9820  Weight:  1.00
Name: mt-bud37         Len:   536  Check: 8525  Weight:  1.00

//
                   1                                                    50
```

```
Asphondylia_ tcgtttaaat aatataagat tttgactatt acctccatca ttaactattt
Asphondylia_ tcgccttaat aatataagat tttgactttt acctccatca ttaacaattt
Asphondylia_ tcgattaaat aatataagat tttgacttct tcctccatca ttaactatcc
mj-blister56 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~TCAATTAT
mo-blister76 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GTGT GATCAAAACA
mt-blister36 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~CTTAAG
  mjk-bulb45 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~C AAAACATATA
   mj-bulb55 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~CAT TCTACACTAT
   mm-bulb25 ~~~~~TTACA TAAATTATAT TTCCCCAGGG TATGTTTTTT TTTATTTTTA
 mt-bullet39 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~CTATT AAGACCATCA CTTTTAATAA
 mj-bullet59 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~G GGTTATTTTT TTGTTTCACC
 mz-bullet19 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~CT ACCACCATCA ATTCTAATTA
 mm-bullet29 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGGCG ACCACCATCA ATTCTAATTA
 mo-bullet79 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~CTTATT ACCTCCATCA ATTTTAATTC
mjk-bullet49 ~~~~~~~~~~ ~~~~~~TTTA GTTTCGGGTT TCCTCCATCA ATTTTAATTC
 mp-bullet69 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~GG TTCCTCCTCA ATTTTAATTC
   mj-mice51 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~ATT ACCTCCTTCA CTTTCTATTC
  mjk-mice41 ~~~~~~~~~~ ~~~~~~~~~~ ~~~GATTATT ACCTCCTTCA CTTTCTATTC
   mt-mice31 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~TATT ACCTCCTTCA CTTTCTATTC
   mz-mice11 ~~~~~~~~~~ ~~~~~~TTAG ATCCGAGAGG ACCTCCTTCA CTTTCTATTC
   mm-mice21 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~CTTT ACCTCCATCA CTTTCTATTC
   mt-bell32 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~TTACT ACCTCCATCA TTATCTATTC
   mm-bell22 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GGCT ACCTCCATCA TTATCTATTC
  mjk-bell42 ~~~~~~~~~~ ~~~~~~~TAG ATTTGATTCT ACCTCCATCA TTATCTATTC
```

```
     mt-club34 ~~~~~~~~~~ ~~~TATTAAT TTTCCGGGGA ACCTCCATCA TTATCTATTC
     mo-club74 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ACCTCCATCA TTATCTATTC
     mp-club64 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ACCTCCTTCA TTGTCTATTC
     mz-bell12 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GGAT ACCTCCATCA CTATCTATTC
     mk-bird83 ~~~~~~~~~~ ~~~~~~~~~T ACACGGGGCT ACCTCCATCA CTATCTATTC
     mj-club54 ~~~~GGCTAT TTGTAGTATT ATCGAGGACT ACCTCCTTCA TTATCTATTC
    mjk-club44 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~T TTATCTATTC
  mp-spindle68 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~CCCCCTCT TTACCTTATT
  mt-spindle38 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~CTT TAACCTTATT
  mm-spindle28 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~AA AAATTTTATT
  mz-spindle18 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~AATTTTATT
  mj-spindle58 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~TT TAATTTTATT
      mz-bud17 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~TTC AAACTTTATA
      mt-bud37 ~~~~~~~~~~ ~~~~TTTTAA ATACGGGTCT CCCTCCTTCT TTAATATTAC

               51                                                100
  Asphondylia_ tattaataag aagaattatt .gaaaacggg actggaaccg gatgaactat
  Asphondylia_ tattaataag aagaattatt .gaaagaggg acaggaacag gatgaacagt
  Asphondylia_ tattaataag ttcaattatc .gaaagaggg acaggaacag gctgaacaat
  mj-blister56 CTAATTAAGA ATGATTAATA AAAACTGGGA CTGGGGACTG GATGAACAGT
  mo-blister76 TTAATTAAGA AGAATAATAG AAACTTGGAA CTAGGAACTG GATGAACAGT
  mt-blister36 GCATTTGAAG GGCAGCACAA CAGGCGAATC AGGAACATGG TTCGAACAGT
    mjk-bulb45 TTATATAAAG AAAAACTGGT AAAAACAGGA ACAGGAACGG GATGAACTGT
     mj-bulb55 GTTATTAAAG AAAAACTTGT AAAAACAGGA ACAGGAACGG GATGAACTGT
```

mm-bulb25	CCCGGGGATT	TGAATTCTGT	AGAAACAGGA	ACAGGGACAG	GATGAACTGT
mt-bullet39	TTATTTTAAG	AATAATAATT	.GAAATAGGA	ACTGGAACAG	GATGAACTAT
mj-bullet59	GGGTTTTAAG	AATAATAGGG	.GAAAGAGGA	GCTGGAACAG	GATGAACTAT
mz-bullet19	TTCTTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACTAT
mm-bullet29	TTCTTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACTAT
mo-bullet79	TTCTTATAAG	AAGAATAATT	.GAAAAAGGA	ACTGGAACAG	GATGAACTGT
mjk-bullet49	TTCTTATAAG	AAGAATAATT	.GAAAAAGGA	ACTGGAACAG	GATGAACTAT
mp-bullet69	TTCTTATAAG	AAGAATAATT	.GAAAAAGGA	ACTGGAACAG	GATGAACTAT
mj-mice51	TTCTTTTAAG	AAGAATAATT	.GAAAGGGGA	ACTGGAACAG	GATGAACTGT
mjk-mice41	TTCTTTTAAG	AAGAATAATT	.GAAAGGGGA	ACTGGAACAG	GATGAACTGT
mt-mice31	TTCTTTTAAG	AAGAATAATT	.GAAAGGGGA	ACTGGAACAG	GATGAACTGT
mz-mice11	TTCTTTCAAG	AAGAATAATT	.GAAAGGGGA	ACTGGAACAG	GATGAACTGT
mm-mice21	TTCTTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACAGT
mt-bell32	TTATTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACAGT
mm-bell22	TTATTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACAGT
mjk-bell42	TTATTTTAAG	AAGAATAATT	.GAAAGAGGA	ACTGGAACAG	GATGAACAGT
mt-club34	TTATTATAAG	AAGAATAATT	.GAAAGAGGC	ACTGGAACAG	GATGAACAGT
mo-club74	TTATTATAAG	AAGAATAATT	.GAAAGAGGC	ACTGGAACAG	GATGAACAGT
mp-club64	TTATTTTAAG	AAGAATAATT	.GAAAGAGGT	ACTGGAACAG	GATGAACAGT
mz-bell12	TTATTTTAAG	AAGAATAATT	.GAAAGAGGT	ACTGGAACAG	GATGAACAGT
mk-bird83	TTATTTTAAG	AAGAATAATT	.GAAAGAGGT	ACTGGAACAG	GATGAACAGT
mj-club54	TTATTTTAAG	AAGAATAATT	.GAAAGAGGT	ACTGGAACAG	GATGAACAGT
mjk-club44	TTATTTTAAG	AAGAATAATT	AGAAAGAGGA	CTAGGAACAG	GATGAACAGT
mp-spindle68	ATTAATT.AG	AAGAATTATT	.GAAAATGGA	ACAGGAACAG	GATGAACAAT

```
mt-spindle38 ATTAATT.AG AAGAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
mm-spindle28 ATTAA.TAAG AATAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
mz-spindle18 ATTAA.TCAG AATAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
mj-spindle58 ATTAATTCAG AATAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
     mz-bud17 TATTAATTAA AAGAATAGTA .GAAACTGGA TCAGGAACAG GATGAACTAT
     mt-bud37 TTTTAATCAG AAGAAT.GGT AGAAACAGGG ACAGGGACAG GATGAACTGT

             101                                                150
Asphondylia_ ttatcctcct ttatcatcaa ttattgctca taatggaaga tcaactgatt
Asphondylia_ ttatcccccct ttatcctcaa ttattgctca taatagaaga tcaacagatt
Asphondylia_ ttacccccca ttatcatcta ttattgctca taatagaaga tcaactgatt
mj-blister56 TTATCCCCCT CTTTCATCAA CAATTGCTCA TACTGGATCT TCAGTATATT
mo-blister76 CTATCCACCC CTTTCTTCTA TTATTGCACA TACAGGCTCT TCTGTAGATT
mt-blister36 TTACCCTCCA CTTTCATCAA CTATTGCTCA TACAGGATCA TCTGTTGATT
   mjk-bulb45 ATATCCACCA CTTTCATCAA TTATTGCCCA TAATGGAGCA TCTGTTGACT
    mj-bulb55 ATATCCACCA CTTTCATCAA TTATTGCCCA TAATGGAGCA TCTGTTGACT
    mm-bulb25 ATACCCACCA CTCTCATCAA TTATTGCCCA TAATGGTGCG TCTGTTGACT
  mt-bullet39 TTATCCCCCT CTTTCTTCAA TTATAGCACA TAGTAGAGCA TCTGTAGATT
  mj-bullet59 TTACCCCCCT CTTTCTTCAA TTATAGCACA TAAGGGAGCA TCTGTAGACT
  mz-bullet19 TTATCCACCT CTTTCCGCAA TTATTGCACA TAATAGACCA TCTGTTGATC
  mm-bullet29 TTATCCGCCT CTTTCCGCAA TTATTGCACA TAATAGACCA TCTGTTGATC
  mo-bullet79 TTACCCCCCT CTATCTTCAA TTATTGCGCA TAATGGACCA TCTGTTGATC
 mjk-bullet49 TTACCCCCCT CTATCTTCAA TTATTGCACA TAATGGACCA TCTGTTGATC
  mp-bullet69 TTACCCTCCT CTATCTTCAA TTATTGCACA TAATGGACCA TCTGTTGATC
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   mj-mice51 CTACCCACCT CTTTCTTCTA TTATAGCCCA TAATAGATCA TCAGTAGATT
  mjk-mice41 CTACCCACCT CTTTCTTCTA TTATAGCCCA TAATAGATCA TCAGTAGATT
   mt-mice31 CTACCCACCT CTTTCTTCTA TTATAGCCCA TAATAGATCA TCAGTAGATT
   mz-mice11 CTACCCACCT CTTTCTTCTA TTATAGCCCA TAATAGATCA TCAGTAGATT
   mm-mice21 CTACCCACCT CTTTCTTCTA TTATAGCCCA TAATAGATCA TCAGTAGATT
   mt-bell32 ATACCCTCCT CTTTCTTCAA TTATAGCGCA TAATGGATCA TCAGTAGATT
   mm-bell22 ATACCCTCCT CTTTCTTCAA TTATAGCGCA TAATGGATCA TCAGTAGATT
  mjk-bell42 ATACCCTCCT CTTTCTTCAA TTATAGCGCA TAATGGATCA TCAGTAGATT
   mt-club34 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
   mo-club74 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
   mp-club64 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCG TCAGTAGATT
   mz-bell12 TTACCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
   mk-bird83 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
   mj-club54 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
  mjk-club44 TTATCCTCCT CTTTCTTCAA TTATAGCTCA TAATGGATCA TCAGTAGATT
mp-spindle68 TTACCCTCCT CTTTCTTCAA TTATTGCACA TACTAGACCT TCAGTAGACT
mt-spindle38 TTACCCTCCT CTTTCTTCAA TTATTGCACA TACTAGACCT TCAGTAGACT
mm-spindle28 TTATCCCCCC CTTTCTTCTA TTACTGCACA TACTAGAAAT TCAGTAGATT
mz-spindle18 TTATCCCCCC CTTTCTTCTA TTAATGCACA TACTAGAAAT TCAGTAGATT
mj-spindle58 TTACCCCCCC CTTTCTTCTA TTACTGCACA TACTAGAACT TCAGTAGATT
    mz-bud17 TTATCCCCCC TTATCTTCCA TTATTGCTCA TACAAGTTCT TCAGTAGATT
    mt-bud37 ATATCCTCCT CTTTCTTCAT CAATTGCCCA TACTGGCTCA TCAGTTGATT

             151                                                  200
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Asphondylia_ tatcaatctt ttcacttcat atcgcaggaa tttcttctat tttaggagct
Asphondylia_ tatctatttt ttcacttcat attgctggta tctcttctat tttaggagct
Asphondylia_ tatcaatttt ttcattacac attgcaggaa tctcttctat tttaggggca
mj-blister56 TTTCTATTTT TTCTCTTCAT ATTGCTGGAA TTTCTTCTAT TTTAGGAGCA
mo-blister76 TTTCAATTTT TTCACTACAT ATCGCGGGAA TCTCATCCAT TTTGGGGGCG
mt-blister36 TTTCTATTTT TTCACTACAT ATTGCAGGAA TTTCATCAAT CCTAGGAGCA
   mjk-bulb45 TATCATTTTT TTCTCTTCAT ATTGCAGGAA TTTCATCATT TTTAGGAGCA
    mj-bulb55 TATCATTTTT TTCTCTTCAT ATTGCAGGAA TTTCATCATT TTTAGGAGCA
    mm-bulb25 TATCAATTTT TTCTCTCCAT ATTGCAGGAA TCTCATCAAT TTTGGGAGCA
  mt-bullet39 TATCTATTTT TTCACTTCAT ATAGCAGGAA TTTCATCAAT TTTAAGATCT
  mj-bullet59 TATCTATTTT TTCACTTCAT ATAGCAGGAA TTTCATCAAT TTTAAGATCT
  mz-bullet19 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
  mm-bullet29 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
  mo-bullet79 TATCTATTTT TTCACTACAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
mjk-bullet49 TTTCTATTTT TTCACTACAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
  mp-bullet69 TATCTATTTT TTCACTACAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
    mj-mice51 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT CTTAGGAGCT
   mjk-mice41 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT CTTAGGAGCT
    mt-mice31 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT CTTAGGAGCT
    mz-mice11 TATCTATTTT TTCACTTCAT ATTGCAGGAA TTTCATCAAT CTTAGGAGCT
    mm-mice21 TATCTATTTT TTCGCTTCAT ATTGCAGGAA TTTCATCAAT CTTAGGAGCT
    mt-bell32 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCA
    mm-bell22 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCA
   mjk-bell42 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCA
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     mt-club34 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
     mo-club74 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
     mp-club64 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
     mz-bell12 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
     mk-bird83 TATCTATTTT TTCCCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
     mj-club54 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
    mjk-club44 TATCTATTTT TTCTCTTCAT ATTGCAGGAA TTTCATCAAT TTTAGGAGCT
  mp-spindle68 TCTCTATTTT TTCCCTTCAT ATTGCTGGAA TTTCCTCTAT CTTAGGAGCA
  mt-spindle38 TCTCTATTTT TTCCCTTCAT ATTGCTGGAA TTTCCTCTAT CTTAGGAGCA
  mm-spindle28 TTTCAATTAT TTCCCTCCAT ATAGCTGGAA TTTCCTCTAT TTTAGGAGCA
  mz-spindle18 TTTCAATTAT TTCCCTCCAT ATAGCTGGAA TTTCCTCTAT TTTAGGAGCA
  mj-spindle58 TTTCTATTTT TTCCCTCCAT ATTGCTGGAA TTTCCTCTAT TTTAGGAGCA
      mz-bud17 TTTCATTTTT TTCACTTCAT ATAGCTGGAA TTTCTTCTAT TTTAGGAGCT
      mt-bud37 TCTCAATTTT TTCTTTACAT ATTGCTGGTA TTTCCTCAAT TTTAGGGGCA

               201                                              250
  Asphondylia_ attaatttta ttactacaat tattaacata aaaaataaat ttattaaaat
  Asphondylia_ attaatttta ttactactat cattaatata aaaaataaat ttattaaatt
  Asphondylia_ attaatttta ttactacaat tattaatata aaaaataaat ttattaaatt
  mj-blister56 ATTAATTTTA TTTCAACTAT ATTTAATATA AAAATTAAAT TTTTAAAATT
  mo-blister76 ATTAATTTTA TTTCAACTAT ATTAAATATA AAAATTAAAT TTTTAAAATT
  mt-blister36 ATTAATTTTA TTTCAACTAT ATTTAATATA AAAATTAAAT TTTTAAAATT
    mjk-bulb45 ATTAATTTTA TTTCAACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
     mj-bulb55 ATTAATTTTA TTTCAACTAT TATAAATATA AAAAATAAAA ATTTAAAATT
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mm-bulb25 ATTAATTTTA TCTCAACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mt-bullet39 ATAAATTTTA TTTCTACAAT TATAAATATA AAAAATATAA ATTTAAAATT
mj-bullet59 ATCAATTTTA TTTCTACAAT TATAAATATA AAAAATATAA ATTTAAAATT
mz-bullet19 ATCAATTTTA TTTCTACAAT TATAAATATA AAAAATAATA ATTTAAATTT
mm-bullet29 ATCAATTTTA TTTCTACAAT TATAAATATA AAAAATAATA ATTTAAATTT
mo-bullet79 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAATA ATTTAAAATT
mjk-bullet49 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAATA ATTTAAAATT
mp-bullet69 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAATA ATTTAAAATT
mj-mice51 ATTAATTTTA TTTCTACGAT TATAAATATA AAAAACAAAA ATTTAAAATT
mjk-mice41 ATTAATTTTA TTTCTACGAT TATAAATATA AAAAACAAAA ATTTAAAATT
mt-mice31 ATTAATTTTA TTTCTACGAT TATAAATATA AAAAACAAAA ATTTAAAATT
mz-mice11 ATTAATTTTA TTTCTACGAT TATAAATATA AAAAACAAAA ATTTAAAATT
mm-mice21 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mt-bell32 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mm-bell22 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mjk-bell42 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mt-club34 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mo-club74 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mp-club64 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mz-bell12 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mk-bird83 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mj-club54 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mjk-club44 ATTAATTTTA TTTCTACAAT TATAAATATA AAAAATAAAA ATTTAAAATT
mp-spindle68 ATTAATTTTA TTTCAACAAT AATTAATATA AAAATTAAAT TTCTAAAGAT

```
mt-spindle38 ATTAATTTTA TTTCAACAAT AATTAATATA AAAATTAAAT TTCTAAAGAT
mm-spindle28 ATTAATTTTA TCTCAACAAT AATTAATATA AAAATTAAAT ATTTAAAAAT
mz-spindle18 ATTAATTTTA TCTCAACAAT AATTAATATA AAAATTAAAT ATTTAAAAAT
mj-spindle58 ATTAATTTTA TCTCAACAAT AATTAATATA AAAATTAAAT ATTTAAAAAT
     mz-bud17 ATTAATTTTA TTTCTACTAT TTTAAATATA AAAATTAAAT TTATTAAAGC
     mt-bud37 ATTAACTTTA TTTCAACAAT ACTAAATATA AAAATTAAAT TTATTAAATT

             251                                                300
Asphondylia_ taatgaatta tcactttttta tctgatcaat tttaattact accattcttt
Asphondylia_ aaatgaactt tcccttttta tttgatcaat tttaattact actgttcttt
Asphondylia_ taatgaaata tcactattta tttgatcaat tctaattaca actattcttt
mj-blister56 CGATCAAATT TCTTTATTTA CATGATCAGT ACTAATTACA GCATTTTTAT
mo-blister76 TGATCAAATT TCTTTATTTA TTTGATCTAT TATAATCACT ACTATCCTTT
mt-blister36 TGACCAAATT TCTTTATTTA CATGATCAGT ATTAATTACT GCATTTCTTT
   mjk-bulb45 TAATAAATTA TCTTTATTTA TTTGATCAAT TTTAATTACA ACTATTTTAT
    mj-bulb55 TAATAAATTA TCTTTATTTA TTTGATCAAT TTTAATTACA ACTATTTTAT
    mm-bulb25 TAATAAATTA TCTTTATTTA TTTGATCAAT TTTAATTACA ACTATTTTAT
  mt-bullet39 TTATGAACTT TCTTTATTCA TTTGATCAAT TCTCATTACA TCAATTTTAT
  mj-bullet59 TTATGAACTT TCTTTATTTA TTTGATCAAT TCTTATTACA TCAATTTTAT
  mz-bullet19 TAATAAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
  mm-bullet29 TAATAAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
  mo-bullet79 TAATAAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
 mjk-bullet49 TAATAAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
  mp-bullet69 TAATAAACTT TCTTTATTTA TTTGATCAAT TTTTATTACA ACAATTTTAT
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mj-mice51	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATCACA	ACAATTTTAT
mjk-mice41	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATCACA	ACAATTTTAT
mt-mice31	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATCACA	ACAATTTTAT
mz-mice11	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATCACA	ACAATTTTAT
mm-mice21	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATTACA	ACAATTTTAT
mt-bell32	TAATGAACTT	TCTTTATTCA	TTTGATCAAT	TTTTATTACA	ACAATCTTAT
mm-bell22	TAATGAACTT	TCTTTATTCA	TTTGATCAAT	TTTTATTACA	ACAATCTTAT
mjk-bell42	TAATGAACTT	TCTTTATTCA	TTTGATCAAT	TTTTATTACA	ACAATCTTAT
mt-club34	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATTACA	ACAATTTTAT
mo-club74	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATTACA	ACAATTTTAT
mp-club64	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATTACT	ACAATTTTAT
mz-bell12	TAATGAACTT	TCTTTATTCA	TTTGATCAAT	TTTTATTACT	ACAATTTTAT
mk-bird83	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATTACA	ACAATTTTAT
mj-club54	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATTACT	ACAATTTTAT
mjk-club44	TAATGAACTT	TCTTTATTTA	TTTGATCAAT	TTTTATTACT	ACAATTTTAT
mp-spindle68	AGATCAAATA	TCATTATTTA	TTTGATCTAT	TTTAATTACA	ACGATCTTAT
mt-spindle38	AGATCAAATA	TCATTATTTA	TTTGATCTAT	TTTAATTACA	ACAATCTTAT
mm-spindle28	AGATCAAATA	TCACTTTTTA	TTTGATCTAT	TTATATTACA	ACAATTCTTT
mz-spindle18	AGATCAAATA	TCACTTTTTA	TTTGATCTAT	TTATATTACA	ACAATTCTTT
mj-spindle58	AGATCAAATA	TCATTTTTTA	TTTGATCTAT	TTATATTACA	ACAATTCTTT
mz-bud17	TAATCAAATT	TCATTATTTA	TTTGATCAAT	TATTATTACA	GCTATTTTAC
mt-bud37	TGATCAAATT	TCTTTATTCA	CATGATCAGT	ATTAATTACT	GCCATTCTTT

301 350

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Asphondylia_ tacttttatc tttaccagtt cttgcaggag caatcactat actaattact
Asphondylia_ tacttttatc acttcctgta cttgcaggag caattactat attattaact
Asphondylia_ tattattatc attacctgta ttagctggag caattactat attattaact
mj-blister56 TATTATTATC ATTACCAGTT TTAGCTGGAG CTATTACAAT ATTATTAATA
mo-blister76 TACTCCTTTC TTTACCTATT TTAGCAGGAG CTATTACTAT ACTTTTAACT
mt-blister36 TATTATTATC TTTACCAGTA TTAGCAGGAG CAATTACAAT ATTATTAATA
  mjk-bulb45 TACTTTTATC ATTACCTGTT TTGGCCGGAG CTATTACAAT ATTATTAACA
   mj-bulb55 TACTTTTATC ATTACCTGTT TTGGCCGGAG CTATTACAAT ATTATTAACA
   mm-bulb25 TACTTTTATC ATTACCTGTT TTGGCCGGAG CTATTACAAT ATTATTAACA
 mt-bullet39 TATTACTATC ATTACCAGTC TTAGCTGGAG CAATTACAAT ATTATTAACT
 mj-bullet59 TATTACTATC ATTACCAGTA TTAGCTGGAG CAATCACAAT ATTATTAACT
 mz-bullet19 TATTACTATC CTTACCAGTT TTAGCTGGAG CAATCACAAT ATTGCTAACT
 mm-bullet29 TATTACTATC CTTACCAGTT TTAGCTGGAG CAATCACAAT ATTACTAACT
 mo-bullet79 TATTATTATC TTTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACT
mjk-bullet49 TATTATTATC TTTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACT
 mp-bullet69 TATTATTATC TTTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACT
   mj-mice51 TATTATTATC TTTACCAGTA TTAGCTGGAG CAATTACAAT ATTATTAACT
  mjk-mice41 TATTATTATC TTTACCAGTA TTAGCTGGAG CAATTACAAT ATTATTAACT
   mt-mice31 TATTATTATC TTTACCAGTA TTAGCTGGAG CAATTACAAT ATTATTAACT
   mz-mice11 TATTATTATC TTTACCAGTA TTAGCTGGAG CAATTACAAT ATTATTAACT
   mm-mice21 TATTATTATC TTTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACT
   mt-bell32 TATTATTATC ATTACCAGTT TTAGCAGGAG CAATTACAAT ATTATTAACA
   mm-bell22 TATTATTATC ATTACCAGTT TTAGCAGGAG CAATTACAAT ATTATTAACA
  mjk-bell42 TATTATTATC ATTACCAGTT TTAGCAGGAG CAATTACAAT ATTATTAACA
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    mt-club34 TATTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACA
    mo-club74 TATTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACA
    mp-club64 TATTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACA
    mz-bell12 TGTTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACA
    mk-bird83 TATTACTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ATTATTAACA
    mj-club54 TATTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ACTATTAACA
   mjk-club44 TATTATTATC ATTACCAGTT TTAGCTGGAG CAATTACAAT ACTATTAACA
mp-spindle68 TAATTATTTC TTTACCTGTT TTAGCAGGAG CAATTACTAT ATTATTAACA
mt-spindle38 TAATTATTTC TTTACCTGTT TTAGCAGGAG CAATTACTAT ATTATTAACA
mm-spindle28 TATTACTTGC ATTACCTGTT TTAGCAGGAG CAATTACTAT ACTACTAACA
mz-spindle18 TATTACTTGC ATTACCTGTT TTAGCAGGAG CAATTACTAT ACTACTAACA
mj-spindle58 TATTACTTGC TTTACCTGTT TTAGCAGGAG CAATTACTAT ACTACTAACA
      mz-bud17 TTCTTCTTTC ATTACCTGTT TTAGCTGGAG CAATTACTAT ATTATTAACT
      mt-bud37 TATTATTATC ACTACCAGTT CTAGCCGGAG CAATTACTAT ACTTCTTACT

             351                                              400
Asphondylia_ gaccgaaact taaatacttc atttttttgat cctataggag ggggtgaccc
Asphondylia_ gatcgaaata ttaatactac atttttttgac ccaataggag gaggagatcc
Asphondylia_ gatcgaaata ttaatacatc tttttttgat cctataggag ggggtgatcc
mj-blister56 GATCGAAACT TAAATACATC ATTCTTTGAT CCTATAGGAG GAGGAGATCC
mo-blister76 GATCGAAATT TAAATACATC ATTTTTTGAC CCCATAGGAG GAGGAGATCC
mt-blister36 GACCGTAATT TAAATACATC ATTTTTTGAT CCAATAGGAG GAGGAGATCC
   mjk-bulb45 GATCGAAATA TAAATACATC TTTTTTTGAC CCACTCGGAG GAGGAGATCC
     mj-bulb55 GATCGAAATA TAAATACATC ATTTTTTGAC CCACTCGGAG GAGGAGATCC
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mm-bulb25	GATCGAAATA	TAAATACATC	ATTTTTTGAC	CCACTCGGAG	GAGGAGACCC
mt-bullet39	GATCGAAATT	TAAATACTTC	TTTTTTTGAT	CCTATTGGAG	GAGGAGATCC
mj-bullet59	GATCGAAATT	TAAATACTTC	TTTTTTTGAT	CCTATTGGAG	GAGGTGATCC
mz-bullet19	GATCGAAATT	TAAATACATC	TTTTTTCGAT	CCATTAGGAG	GTGGAGATCC
mm-bullet29	GATCGAAATT	TAAATACATC	TTTTTTCGAT	CCATTAGGAG	GTGGAGATCC
mo-bullet79	GATCGAAATT	TAAATACATC	TTTCTTTGAC	CCATTAGGAG	GTGGAGATCC
mjk-bullet49	GATCGAAATT	TAAATACATC	TTTCTTTGAC	CCATTAGGAG	GTGGAGATCC
mp-bullet69	GATCGAAATT	TAAATACATC	TTTCTTTGAC	CCATTAGGAG	GTGGAGATCC
mj-mice51	GATCGAAATT	TAAATACTTC	ATTTTTTGAC	CCACTAGGAG	GAGGAGATCC
mjk-mice41	GATCGAAATT	TAAATACTTC	ATTTTTTGAC	CCACTAGGAG	GAGGAGATCC
mt-mice31	GATCGAAATT	TAAATACTTC	ATTTTTTGAC	CCACTAGGAG	GAGGAGATCC
mz-mice11	GATCGAAATT	TAAATACTTC	ATTTTTTGAC	CCACTAGGAG	GAGGAGATCC
mm-mice21	GATCGAAATT	TAAATACTTC	ATTTTTTGAT	CCTCTAGGAG	GGGGAGATCC
mt-bell32	GATCGAAATT	TAAATACTTC	TTTTTTTGAT	CCATTAGGAG	GAGGAGATCC
mm-bell22	GATCGAAATT	TAAATACTTC	TTTTTTTGAT	CCATTAGGAG	GAGGAGATCC
mjk-bell42	GATCGAAATT	TAAATACTTC	TTTTTTTGAT	CCATTAGGAG	GAGGAGATCC
mt-club34	GATCGAAATT	TAAACACTTC	TTTTTTTGAT	CCATTAGGAG	GAGGAGATCC
mo-club74	GATCGAAATT	TAAACACTTC	TTTTTTTGAT	CCATTAGGAG	GAGGAGATCC
mp-club64	GATCGAAATT	TAAACACTTC	TTTTTTTGAT	CCATTAGGAG	GAGGAGATCC
mz-bell12	GATCGAAATT	TAAATACTTC	TTTTTTTGAT	CCATTAGGAG	GAGGAGATCC
mk-bird83	GATCGAAATT	TAAATACTTC	TTTTTTTGAT	CCATTAGGAG	GAGGAGATCC
mj-club54	GATCGAAATT	TAAACACTTC	TTTTTTTGAT	CCATTAGGAG	GAGGAGATCC
mjk-club44	GATCGAAATT	TAAACACTTC	TTTTTTTGAT	CCATTAGGAG	GAGGAGATCC
mp-spindle68	GATCGAAATC	TAAACACATC	ATTTTTCGAT	CCTATAGGAG	GAGGAGATCC

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mt-spindle38 GATCGAAATC TAAACACATC ATTTTTCGAT CCTATAGGAG GAGGAGATCC
mm-spindle28 GATCGAAATC TAAATACATC ATTTTTTGAC CCAATAGGAG GAGGAGATCC
mz-spindle18 GATCGAAATC TAAATACATC ATTTTTTGAC CCAATAGGAG GAGGAGATCC
mj-spindle58 GATCGAAATC TAAATACATC ATTTTTTGAC CCAATAGGAG GAGGAGATCC
     mz-bud17 GATCGAAATT TAAATACATC ATTTTTTGAT CCTATAGGAG GAGGTGACCC
     mt-bud37 GATCGAAATT TAAATACATC TTTTTTTGAT CCAATAGGGG GAGGAGATCC


             401                                              450
Asphondylia_ aattctttat caacatttat tttgattttt tggtcatcct ~~~~~~~~~~
Asphondylia_ aattctttat caacatttat tttgattttt tggtcatcct ~~~~~~~~~~
Asphondylia_ tattctttat caacatttat tttgattttg cggtcaccca ~~~~~~~~~~
mj-blister56 AATTTTATAT CAACATTTAT TTTGATTTTT TGGACATCCA GAATTTTATA
mo-blister76 ATTTTTATAT CAACACTTAT TTTGATTTTT TGGCCACCCT GAATTTTATA
mt-blister36 AATTTTATAT CAACATTTAT TTTGATTTTT TGGTCACCCA GAAGTTTATA
   mjk-bulb45 AATTTTATAT CAACATTTAT TTTGATTTTT TGGACATCCA GAATTTTATA
    mj-bulb55 AATTTTATAT CAACATTTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
    mm-bulb25 AATTTTATAC CAACATTTAT TTTGATTTTT TGGACACCCA GAAGTTTATA
  mt-bullet39 TATTCTATAT CAACATCTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
  mj-bullet59 TATTCTTTAT CAACATCTAT TTTGATTTTT TGGACATCCA GAAGTTTATA
  mz-bullet19 AATTTTATAT CAACATTTAT TTTGATTTTT TGGCCACCCA GAAGTTTATA
  mm-bullet29 AATTTTATAT CAACATTTAT TTTGATTTTT TGGTCACCCA GAAGTTTATA
  mo-bullet79 AATTTTATAT CAACATTTAT TTTGATTTTT TGGTCATCCA GAAGTTTATA
mjk-bullet49 AATTTTATAT CAACATTTAT TTTGATTTTT TGGTCATCCA GAAGTTTATA
```

mp-bullet69	AATTTTATAT	CAACATTTAT	TTTGATTTTT	TGGTCATCCA	GAAGTTTATA
mj-mice51	AATTCTTTAT	CAACATTTAT	TTTGATTCTT	TGGACATCCT	GAAGTTTATA
mjk-mice41	AATTCTTTAT	CAACATTTAT	TTTGATTCTT	TGGACATCCT	GAAGTTTATA
mt-mice31	AATTCTTTAT	CAACATTTAT	TTTGATTCTT	TGGACATCCT	GAAGTTTATA
mz-mice11	AATTCTTTAT	CAACATTTAT	TTTGATTCTT	TGGACATCCT	GAAGTTTATA
mm-mice21	AATTCTTTAT	CAACATTTAT	TTTGATTCTT	TGGACATCCT	GAAGTTTATA
mt-bell32	AATTCTTTAT	CAACATTTAT	TTTGATTTTT	TGGACATCCA	GAAGTTTATA
mm-bell22	AATTCTTTAT	CAACATTTAT	TTTGATTTTT	TGGACATCCA	GAAGTTTATA
mjk-bell42	AATTCTTTAT	CAACATTTAT	TTTGATTTTT	TGGACATCCA	GAAGTTTATA
mt-club34	AATTCTTTAT	CAACATTTAT	TCTGATTTTT	TGGGCATCCA	GAAGTTTATA
mo-club74	AATTCTTTAT	CAACATTTAT	TCTGATTTTT	TGGGCATCCA	GAAGTTTATA
mp-club64	AATTCTTTAT	CAACATTTAT	TTTGATTTTT	CGGACATCCA	GAAGTTTATA
mz-bell12	GATTCTTTAT	CAACATTTAT	TTTGATTTTT	TGGACATCCA	GAAGTTTATA
mk-bird83	AATTCTCTAT	CAACATTTAT	TCTGATTTTT	TGGACATCCA	GAAGTTTATA
mj-club54	AATTCTTTAT	CAACATTTAT	TTTGATTTTT	CGGACATCCA	GAAGTTTATA
mjk-club44	AATTCTTTAT	CAACATTTAT	TTTGATTTTT	CGGACATCCA	GAAGTTTATA
mp-spindle68	TATTTTATAT	CAACATTTAT	TTTGATTTTT	TGGTCATCCA	GAAGTTTATA
mt-spindle38	TATTTTATAT	CAACATTTAT	TTTGATTTTT	TGGTCATCCA	GAAGTTTATA
mm-spindle28	TATTTTATAT	CAACACTTAT	TTTGATTTTT	TGGACACCCA	GAATTTTATA
mz-spindle18	TATTTTATAT	CAACACTTAT	TTTGATTTTT	TGGACACCCA	GAAGTTTATA
mj-spindle58	TATTTTATAT	CAACACTTAT	TTTGATTTTT	TGGACACCCA	GAAGTTTATA
mz-bud17	AATTCTGTAT	CAACATTTAT	TTTGATTTTT	TGGACATCCA	GAAGTTTATA
mt-bud37	AGTATTATAC	CAACATTTAT	TTTGATTCTT	TGGTCATCCT	GAAGTTTATA

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             451                                                500
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mj-blister56 TTTTAATTGT TACCGGGA~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mo-blister76 TTTTAATTGT TACCGGGATG GGCCTGGTGG GTTATTTTGA ACCAATTTTT
mt-blister36 TTTTAATTTT ACCGGGATTT ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mjk-bulb45 TTTTAATTTT ACCGGGA~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mj-bulb55 TTTTAATTTT ACCGGGA~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mm-bulb25 TTTTATTTTT ACCGGGAGGA AGTTTAT~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mt-bullet39 TTTTATTTTT TCCGGGGGGG ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mj-bullet59 TTTTAATTTT ACCGGG~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mz-bullet19 TTTTATTTTT TCCCCGGGGG G~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mm-bullet29 TTTTAATTTT ACCGGGGG~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mo-bullet79 TTTTAATTTT TTCCGGGG~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mjk-bullet49 TTTTATTTTT TACCGGGGA~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mp-bullet69 TTTTAATTTT TACCGGGGGG GG~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mj-mice51 TTTTAATTTT TACCGGGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mjk-mice41 TTTTAATTTT TCCCGGGGGA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mt-mice31 TTTTAATTTT TCCGGGGAG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mz-mice11 TTTATTTTTT TCCCCGGGGG A~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mm-mice21 TTTTATTTTT ACCGGGGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mt-bell32 TTTTAATTTT CCCCGGGG~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mm-bell22 TTTTAATTTT GCCCGGGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
```

```
  mjk-bell42 TTTAATTTTT TCCCCGGGGG A~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mt-club34 TTTTAATTTT TTCCGGGGA~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mo-club74 TTTTAATTTT ACCGGGGAN~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mp-club64 TTTTAATTTT ACCGGGA~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mz-bell12 TTTTATTTTT TTCCCGGGGG G~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mk-bird83 TTTTAATTTT TCCCCGGGGA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mj-club54 TTTTATTTT~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mjk-club44 TTTTAATTTT ACCGGG~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mp-spindle68 TTTTAATTTT TACCGGGGA~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mt-spindle38 TTTTATTT~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mm-spindle28 TTTTAATTTT ACCGGG~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mz-spindle18 TTTTAATTTT ACCGGG~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mj-spindle58 TTTTAATTTT ACCGGG~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mz-bud17 TTTTATTTTT TACGGGG~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mt-bud37 TTTAATTTTT TCCCGGGGA~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

             501                                  536
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
 mj-blister56 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mo-blister76 CGGTTCTTGG GGAGGGCTAT ATCAGGGGAT ACAACA
mt-blister36 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
   mjk-bulb45 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
```

mj-bulb55 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mm-bulb25 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mt-bullet39 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mj-bullet59 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mz-bullet19 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mm-bullet29 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mo-bullet79 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mjk-bullet49 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mp-bullet69 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mj-mice51 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mjk-mice41 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mt-mice31 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mz-mice11 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mm-mice21 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mt-bell32 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mm-bell22 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mjk-bell42 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mt-club34 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mo-club74 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mp-club64 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mz-bell12 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mk-bird83 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mj-club54 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mjk-club44 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~

```
mp-spindle68 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mt-spindle38 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mm-spindle28 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mz-spindle18 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
mj-spindle58 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
      mz-bud17 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
      mt-bud37 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~
```

Appendix 5. 12s Gene Genetic Distances

Genetic Distances

Calculated over: 65 to 399
Considering all base positions
Correction method: Kimura 2-parameter
Distances are: estimated number of substitutions per 100 bases
Symmatrix version 1
Number of matrices: 1

//
Matrix 1, dimension: 31

Key for column and row indices:

1 mt-bud37
2 mz-bud17
3 mz-spindle18
4 mm-spindle28
5 mj-spindle58
6 mp-spindle68
7 Asphondylia_sphaera_12s
8 Asphondylia_itoi__12s
9 Asphondylia_gennadii_12s
10 mm-bulb25

11 mj-bulb55
12 mz-bulb15
13 mjk-mice41
14 mt-mice31
15 mj-mice51
16 mz-mice11
17 mm-mice21
18 mj-club54
19 mz-bell12
20 mt-bell32
21 mjk-bell42
22 mm-bell22
23 mo-club74
24 mt-club34
25 mt-bullet39
26 mj-bullet59
27 mz-bullet19
28 mm-bullet29
29 mp-bullet69
30 mjk-bullet49
31 mo-bullet79

Matrix 1: Part 1

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.00	0.61	27.91	27.48	30.17	28.66	48.58	48.67	51.32	53.34	53.04	50.64
2		0.00	27.13	26.71	29.38	27.88	46.86	47.72	50.34	52.23	51.89	50.12
3			0.00	0.31	6.76	8.12	54.47	43.63	45.00	58.02	55.85	60.61
4				0.00	6.42	7.77	53.91	43.12	44.47	57.44	55.30	60.03
5					0.00	5.76	53.61	44.67	45.60	61.93	57.17	60.61
6						0.00	53.39	44.65	44.22	59.14	55.29	57.52
7							0.00	15.89	18.33	47.81	46.74	48.44
8								0.00	14.88	41.25	40.05	46.41
9									0.00	43.28	44.06	47.67
10										0.00	5.83	16.68
11											0.00	12.19
12												0.00
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												

23	
24	
25	
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27	
28	
29	
30	
31	

Matrix 1: Part 2

	13	14	15	16	17	18	19	20	21	22	23	24
1	38.53	38.60	43.64	44.44	41.45	39.94	41.86	41.43	41.93	42.11	41.94	43.33
2	38.08	38.20	42.63	43.42	40.47	38.96	40.86	40.44	40.93	41.11	40.94	42.31
3	44.64	44.39	50.74	50.57	49.07	42.60	45.17	47.05	47.56	47.27	43.03	45.24
4	44.11	43.87	50.18	50.02	48.54	42.60	45.17	47.05	47.56	47.27	43.03	45.24
5	46.36	43.58	48.93	48.70	47.80	44.69	46.04	47.42	47.95	47.64	46.43	48.75
6	44.53	42.44	48.47	49.37	46.62	45.69	47.05	48.45	48.97	48.67	46.13	47.12
7	41.13	33.58	34.66	37.38	36.75	35.41	39.30	38.51	39.67	39.26	39.80	40.75
8	38.40	31.66	33.24	35.93	32.20	34.11	36.46	34.54	35.65	35.24	32.18	35.19
9	38.46	32.72	34.63	36.20	34.25	34.05	37.84	37.14	38.27	37.87	35.04	36.97
10	35.43	31.93	36.98	38.15	31.49	36.67	39.92	40.05	40.53	40.81	36.35	37.13

11	33.06	29.70	33.62	34.93	29.31	36.35	38.62	38.89	39.37	39.65	36.00	36.82
12	25.56	28.90	40.01	40.80	37.35	41.91	42.83	43.08	43.57	43.90	42.16	40.67
13	0.00	0.64	12.50	12.91	12.11	21.70	22.26	24.23	24.69	25.22	22.72	21.48
14		0.00	4.61	5.31	7.38	17.51	18.84	18.58	18.99	19.49	18.02	16.85
15			0.00	3.96	8.80	18.33	19.68	20.67	21.09	21.61	18.84	20.23
16				0.00	10.29	20.06	21.45	22.49	22.91	23.46	19.78	21.19
17					0.00	16.14	17.85	19.20	20.06	20.11	17.03	17.53
18						0.00	2.54	5.55	6.58	6.25	2.89	3.21
19							0.00	7.63	8.68	8.35	4.87	4.54
20								0.00	0.95	0.63	7.62	7.97
21									0.00	1.59	8.32	9.03
22										0.00	8.34	8.70
23											0.00	2.89
24												0.00
25												
26												
27												
28												
29												
30												
31												

Matrix 1: Part 3

	25	26	27	28	29	30	31
1	45.37	44.51	40.41	40.41	46.72	47.06	44.95
2	44.35	43.47	38.84	38.84	45.05	45.40	43.31
3	50.98	50.33	44.27	44.48	47.73	48.26	46.95
4	50.44	49.80	43.75	43.95	47.20	47.73	46.42
5	49.11	48.38	43.31	43.51	48.02	48.02	47.26
6	46.64	45.16	39.59	39.76	45.37	45.88	44.63
7	37.42	35.21	31.72	31.72	34.54	34.88	33.01
8	34.02	32.58	31.52	31.52	30.31	31.20	30.31
9	35.94	33.69	29.54	29.54	31.26	31.71	31.84
10	34.71	34.24	30.05	29.67	32.17	32.17	31.70
11	30.05	30.04	28.54	28.16	28.63	28.63	28.19
12	35.16	35.69	37.13	36.74	34.07	34.12	34.12
13	25.73	27.13	27.62	27.23	25.97	25.97	26.41
14	20.44	21.67	21.17	20.80	20.65	21.06	21.06
15	21.21	22.01	21.52	21.15	21.01	21.84	21.42
16	22.59	23.43	21.67	21.30	21.98	22.83	22.40
17	18.14	18.02	18.77	18.40	17.84	18.64	18.24
18	21.30	20.78	18.51	18.15	20.51	20.44	20.05
19	23.15	22.64	21.13	20.77	22.34	22.26	21.86
20	24.62	24.57	22.66	22.29	22.55	22.47	22.06
21	25.97	25.51	23.58	23.21	23.46	23.37	22.96
22	25.60	25.57	23.63	23.26	23.50	23.41	22.59

23	24.07	22.59	20.77	20.40	21.13	21.06	20.66
24	24.65	23.69	21.75	21.38	22.15	22.06	21.66
25	0.00	2.61	12.38	12.43	13.27	13.63	12.88
26		0.00	11.99	12.03	11.79	12.15	12.16
27			0.00	-0.00	12.24	12.62	12.24
28				0.00	12.29	12.66	12.29
29					0.00	0.95	1.92
30						0.00	2.25
31							0.00

```
#NEXUS

begin trees;
utree Tree_1 = (((((((((('mt-bud37':0.85,'mz-bud17':-0.24):11.77,(('mz-spindle18'
:0.35,'mm-spindle28':-0.04):3.47,('mj-spindle58':3.46,'mp-spindle68'
:2.30):0.76):12.45):14.49,(('Asphondylia_sphaera_12s':9.56,
'Asphondylia_itoi__12s':6.33):0.58,'Asphondylia_gennadii_12s'
:8.08):11.26):1.89,(('mm-bulb25':3.94,'mj-bulb55':1.89):2.58,
'mz-bulb15':8.94):17.20):1.51,((('mt-bullet39':1.53,'mj-bullet59'
:1.08):5.68,(('mp-bullet69':0.35,'mjk-bullet49':0.60):0.74,
'mo-bullet79':0.87):4.59):0.56,('mz-bullet19':0.12,'mm-bullet29'
:-0.12):5.42):2.60):2.55,((('mjk-mice41':2.83,'mt-mice31'
:-2.19):3.80,('mj-mice51':1.34,'mz-mice11':2.62):2.74):1.78,
'mm-mice21':3.45):3.60):7.33,(('mt-bell32':-0.08,'mm-bell22'
:0.71):0.11,'mjk-bell42':0.85):4.41):0.87,('mo-club74':1.35,
'mt-club34':1.54):0.85):0.31,'mj-club54':0.36):1.09,'mz-bell12'
:1.09):0.00;
endblock;
```

```
Symbol comparison table: pileupdna.cmp  CompCheck: 6876

                   GapWeight: 5
             GapLengthWeight: 1

PileUp  MSF: 423  Type: N  June 13, 2007 19:31  Check: 2881 ..

 Name: mt-bud37         Len:   423  Check: 4335  Weight:  1.00
 Name: mz-bud17         Len:   423  Check: 8697  Weight:  1.00
 Name: mz-spindle18     Len:   423  Check: 3515  Weight:  1.00
 Name: mm-spindle28     Len:   423  Check: 4247  Weight:  1.00
 Name: mj-spindle58     Len:   423  Check: 6862  Weight:  1.00
 Name: mp-spindle68     Len:   423  Check: 8524  Weight:  1.00
 Name: Asphondylia_sphaera_12s  Len:   423  Check: 7909  Weight:  1.00
 Name: Asphondylia_itoi__12s  Len:   423  Check:  932  Weight:  1.00
 Name: Asphondylia_gennadii_12s  Len:   423  Check: 7522  Weight:  1.00
 Name: mm-bulb25        Len:   423  Check: 4012  Weight:  1.00
 Name: mj-bulb55        Len:   423  Check: 1299  Weight:  1.00
 Name: mz-bulb15        Len:   423  Check: 9622  Weight:  1.00
 Name: mjk-mice41       Len:   423  Check: 2153  Weight:  1.00
 Name: mt-mice31        Len:   423  Check: 1619  Weight:  1.00
 Name: mj-mice51        Len:   423  Check: 4845  Weight:  1.00
 Name: mz-mice11        Len:   423  Check: 5135  Weight:  1.00
 Name: mm-mice21        Len:   423  Check: 3616  Weight:  1.00
```

```
 Name: mj-club54        Len:   423  Check: 2388  Weight:  1.00
 Name: mz-bell12        Len:   423  Check: 3472  Weight:  1.00
 Name: mt-bell32        Len:   423  Check: 2621  Weight:  1.00
 Name: mjk-bell42       Len:   423  Check: 7095  Weight:  1.00
 Name: mm-bell22        Len:   423  Check: 4975  Weight:  1.00
 Name: mo-club74        Len:   423  Check: 5971  Weight:  1.00
 Name: mt-club34        Len:   423  Check: 7708  Weight:  1.00
 Name: mt-bullet39      Len:   423  Check: 2217  Weight:  1.00
 Name: mj-bullet59      Len:   423  Check: 4263  Weight:  1.00
 Name: mz-bullet19      Len:   423  Check: 3254  Weight:  1.00
 Name: mm-bullet29      Len:   423  Check:  866  Weight:  1.00
 Name: mp-bullet69      Len:   423  Check: 4332  Weight:  1.00
 Name: mjk-bullet49     Len:   423  Check: 9049  Weight:  1.00
 Name: mo-bullet79      Len:   423  Check: 9826  Weight:  1.00

//
             1                                                  50
    mt-bud37 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ CTTAAATTAG
    mz-bud17 ~~~~~~~~~~ ~~~~AGAAAA TAACAAGACT GGGCATATGT ACATATTTTT
mz-spindle18 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGTGC GGGCATTTGT ACATATTATT
mm-spindle28 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGTGC GGGCATTTGT .CATATTATT
mj-spindle58 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGTGC GGGCATTTGT ACATATTATT
mp-spindle68 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~C GGGCATTTGT ACATATTATT
Asphondylia_ ~~~tttaaaa ttaattaaaa gcgacgggca atatgtatat attattttaa
```

```
Asphondylia_ ~~~tttaaaa ttaattaaaa gcgacgggca atatgtatat attattttaa
Asphondylia_ ~~~tttaaaa ttaattaaaa gcgacgggcg atatgtatgt attactttta
   mm-bulb25 ~~~~~~~~~~ ~~~~~~~~~~ ~CCGATTGGG AAATGAATAT ATTAAAAATA
   mj-bulb55 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mz-bulb15 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~NNGGGG GGGGCNCGNT GCCGGTNCAG
  mjk-mice41 ~~~~~~~~~~ ~~~~~~~~TG GCATATGAAT ATATTAATAA AATAAATTTA
   mt-mice31 ~~~~~~~~~~ ~~~~~~~~~G GCAGATGAAT ATATTAATAA AATAAATTTA
   mj-mice51 ~~~~~~~~~~ ~~~~~~~~GG GCATGGGCAT ATATTAGTAA AATAAATTTA
   mz-mice11 ~~~~~~~~~~ ~~~~~TGTTG GCATTGGAAT ATATTAATAA AATAAATTTA
   mm-mice21 ~~~~~~~~~~ ~~~~~~~~~~ ~~~TATGAAT ATATTAATAA AATAAATTTA
   mj-club54 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GAAT ATATTTATAA TTTAAATTTA
   mz-bell12 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~AATT ATATTTATAA TTTAAATTTA
   mt-bell32 ~~~~~~~~~~ ~~~~~GGTAG GCATATGGAT ATATTTATAA TTTAAATTTA
  mjk-bell42 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~AT ATATTTATAA TTTAAATTTA
   mm-bell22 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~TGAT ATATTTATAA TTTAAATTTA
   mo-club74 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~CTTTATAA TTTAAATTTA
   mt-club34 ~~~~~~~~~~ ~~~~~~~~~~ ~CATATGAAT ATATTTATAA TTTAAATTTA
 mt-bullet39 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~GTAATTA AATTATCTAA
 mj-bullet59 ~~~~~~~ACC GGGAACTAGG GCAATATGAA TATATTTATA ATATATATTA
 mz-bullet19 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~CAAAT AATCATCTGA
 mm-bullet29 ~~~CATCCAA AAAATTCGGC TTTATTAAAA TATATTAATA AAATATATTA
 mp-bullet69 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~AAAAAAAT TATCATATTA
mjk-bullet49 ~~~~~~~GAC TAAATTTAAG GCATATGAAT ATATTAATAA ATATATATTA
 mo-bullet79 AGGAAAAAAT TCTAAATAGT CAATATGAAT ATATTAATAA ATATATATTA
```

```
                51                                           100
       mt-bud37 CATGTCCATT TAATTATAAA ATTTATTATA ATTTAATTTT AAATACCATT
       mz-bud17 TACATTCATT TAATTATAAA ATTTATTATA ATTTAATTTT AAAT.CCATT
   mz-spindle18 AAAAATTATT CAAATTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
   mm-spindle28 AAAAATTATT CAAATTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
   mj-spindle58 AAAAATTATT CAATTTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
   mp-spindle68 AAAAATTATT CAATTTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
   Asphondylia_ aatattaaaa ttttaaacct ataaaatttt aattttaaat ccattttcat
   Asphondylia_ aatattaaat tattaaattt ataataattt aattttaaat ccattttat
   Asphondylia_ aatattaaat atttaaatat ataaatattt aattttaaat ccaatttcat
      mm-bulb25 ATATTAAATT TTAACTATAT ATTAAAATTT AATATTACCC TCCAACGTTT
      mj-bulb55 ~~~~~~~~~~ ~~~~~TATAT ATTAAAATTT AATATTA..A ATCCAACTTT
      mz-bulb15 CTTANNGNTC NTNNGGATAT ATTAACAATT TA.ATATTAA ATCCAACTTT
    mjk-mice41 AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
      mt-mice31 AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
      mj-mice51 AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
      mz-mice11 AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
      mm-mice21 AATATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
      mj-club54 AAATAAATAT TTATTTATAA .TTTAATATT AAATCCAATT TAAAAAACAT
      mz-bell12 AAATAAATAT TTATTTATAA .TTTAATATT AAATCCAATT TTAAAAATAT
      mt-bell32 AAAAAAATAT TTA.TTATAA .TTTAATATT AAATCCAATT TAAAAAATAT
     mjk-bell42 AAAAAAATAT TTA.TTATAA .TTTAATATT AAAGCCAATT TAAAAAATAT
      mm-bell22 AAAAAAATAT TTA.TTATAA .TTTAATATT AAAT.CAATT TAAAAAATAT
```

```
   mo-club74 AAATAAATAT TTA.TTATAA .TTTAAGATT AAATCCAATT TAAAAAATAT
   mt-club34 AAATAAATAT TTA.TTATAA .TTTAATATT AAATCCAATT TAAAAAATAT
 mt-bullet39 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATATAACTTA
 mj-bullet59 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATATAACTTA
 mz-bullet19 AAAAATAATA TATATTAAAA .TTTAATATT AAATCCAACT ACTATAAATT
 mm-bullet29 AAAAATAATA TATATTAAAA .TTTAATATT AAATCCAACT ACTATAAATT
 mp-bullet69 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATTTTAATTT
mjk-bullet49 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATTTTAATTT
 mo-bullet79 AAAAATAATA TATATTATTT .TTTAATATT AAATTCAATT ATTTTAATTT

             101                                              150
    mt-bud37 TTCAAAAATT TATTACAAAA ATTTATTTAA AATTTATATT AATGTATCTC
    mz-bud17 TTCAAAAATT TATTACAAAA ATTTATTTAA AATTTATATT AATGTATCTC
mz-spindle18 TCATAA.TTT TTTTACAAAA ATTAATTCAA AAACATTTTT ATTGTATTTC
mm-spindle28 TCATAA.TTT TTTTACAAAA ATTAATTCAA AAATATTTTT ATTGTATTTC
mj-spindle58 TCATAA.TTT TTTTACAAAA TTTAATTCAA AAATATTTTT ATTGTATTTC
mp-spindle68 TCATAATTTT TTTTACAAAA TTTAATTCAA AAATATTTTT ATTGTATTTC
Asphondylia_ ttaaatatta aaatttaaaa ttcaatttta aaatttattt aatgtatttc
Asphondylia_ ttaaatatta aaatttaaaa tccaatt... aaatttattt aatgtatttc
Asphondylia_ ttaaatatta aaacttaaaa tcc.atatat aaataaattt aatgtatttc
   mm-bulb25 ATTAATATAT AACAATAAAA ATTCTGCAAA AATTAAAATA AATGTATTTC
   mj-bulb55 ATTAATATAT AACAATAAAA ATTCTGCAAA AATTAAAATA AATGTATTTC
   mz-bulb15 ATTAGTATAT AACAATAAAA ATTCTGCAGA ACTTAAAATA AATGTATTTC
  mjk-mice41 ATT....TAC AATAATAATG CTATAT.AAA A..TAATATT AATGTATTTC
```

```
   mt-mice31 ATT....TAC AATAATAATG CTATAT.AAA A..TAATGTT AATGTATTTC
   mj-mice51 ATT....TAC AATAATAATG CTATAT.AAA A..TAGTATT AATGTATTTC
   mz-mice11 ATT....TAC AATAATAATG CTGTAT.AAA A..TAATATT AATGTAGTTC
   mm-mice21 ATT....TAC AATAATAATG CTATATCCAA A..TAAGAAT AATGTATTTC
   mj-club54 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AATGTATTTC
   mz-bell12 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AAGGTATTTC
   mt-bell32 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AATGTATTTC
  mjk-bell42 TTTACAATAT AA.AATCCAT AGAAATTATA A..AATTATT AATGTATTTC
   mm-bell22 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AATGTATTTC
   mo-club74 CTTACAATAT AAGAAGCCAT ACGAATTATA A..AATTATT AATGTATTTC
   mt-club34 CTTACAATAT AA.AATCCAT AAGACTTATA A..AATTATT AAGGTATTTC
 mt-bullet39 TATTACAATA AATAATAATA TAATACCATT A..TAAATCA AATGTATTTA
 mj-bullet59 TATTACAATA AATATAATAA TTA....ATT A..TAAATCA AATGTATTTC
 mz-bullet19 TATTACAATA AATACTAAAT .......AAA A..TAAATTT AATGTATTTC
 mm-bullet29 TATTACAATA AATACTAAAT .......AAA A..TAAATTT AATGTATTTC
 mp-bullet69 ATTACAATAA ATTATTAAAT ATA...AAAT A..TAAATTA AATGTATTTC
mjk-bullet49 ATTACAATAA ATTATTAAAT ATA...AAAT A..TAAATTA AATGTATTTC
 mo-bullet79 ATTACAATAA ATTAATAAAT ATA...AAAT A..TAAATTA AATGTATTTC

             151                                              200
    mt-bud37 A.TTTAAAAT TTAAATATAA AATGAATTAT GATTTGACAT TTTATATT.A
    mz-bud17 A.TTTAAAAT TTAAATATAA AATGAATTAT GATTTGACAT TTTATATT.A
 mz-spindle18 A.TTTTATTT TTATATATTT AATGAATTTT GATTTGACTT TAAATTTAAA
 mm-spindle28 A.TTTTATTT TTATATATTT AATGAATTTT GATTTGACTT TAAATTTAAA
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mj-spindle58 A.TTTTATTT TTATATATTT AATGAATTTT GATTTGACTT CAAATTTAAA
mp-spindle68 A.TTTTAATT TTATATATTT AATGAATTTT GATTTGACTT CTAATCTTAA
Asphondylia_ atttt.aatc ttatactttt aatatattat gatttgaaat aaattaat..
Asphondylia_ attttaaacc ttaaattttt aatatattat gatttgaatt aaattata..
Asphondylia_ atttttaatc ttttatttta aatatattat gatttgaatt aaattaat..
    mm-bulb25 A.TTTAAAAT TTAATTATTA AATATATTAT GATTTGAATT TTGTTATAT.
    mj-bulb55 A.TTTAAAAT TTAATTATTA AATATATTAT GATTTGAATT TTAATATAT.
    mz-bulb15 A.TTTAAAAT TTAATTATTA AATATATTAT GATTTGAATT TTACTATATA
  mjk-mice41 A.TTTAAATC TTAAATATGA AATATATTAT GATTTGAAAT TTA..TTAT.
    mt-mice31 A.TTTAAATC TTAAATATGA AATATATTAT GATTTGAAAT TTA..TTAT.
    mj-mice51 A.TTTAAATC TTAAATATGA AATATATTAT GATTTGAAAT TTA..TTAT.
    mz-mice11 A.TTTAAATC TTAAATATGA AATATATTAT GATGTGAAGT TTA..TTAT.
    mm-mice21 A.TTTAAATC TTAAATATAA AATATATTAT GATTTGAAAT TTATTTTTT.
    mj-club54 A.TTTAAATT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
    mz-bell12 A.TTTAAATT TTAAATATAA AATATATTAG GATTGGAAAT TATTTTAAT.
    mt-bell32 A.TTTAAAAT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
  mjk-bell42 A.TTTAAAAT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
    mm-bell22 A.TTTAAAAT TTAAATATAA AAGATATTAT GATTTGAAAT TATTTTAAT.
    mo-club74 A.TTTAAACT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
    mt-club34 A.TTTAAACT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
  mt-bullet39 A.TTTAAATC TTAAATATAA AATATATTAT AATTTGAAAT TTA.T.TAA.
  mj-bullet59 A.TTTAAATC TTAAATATAA AATATATTAT AATTTGAAAT TTATT.TTA.
  mz-bullet19 A.TCTAAATC TTAAATATAA AATATATTAT GATTTGAATT TTTTTAATA.
  mm-bullet29 A.TCTAAATC TTAAATATAA AATATATTAT GATTTGAATT TTTTTAAT..
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 mp-bullet69 A.TTTAAACC TTAAATATAA AATATATTAT GATTTGAAAT TAATTACTA.
mjk-bullet49 A.TTTAAACC TTAAATATAA AATATATTAT GATTTGAAAT TAATTACTA.
 mo-bullet79 A.TTTAAACC TTAAATATAT AATATATTAT GATTTGAAAT TAATTACTA.

             201                                              250
     mt-bud37 TTCAAATATT TAAATAATAA ATTTTTTAAA AATTATTTTA TGACAACAAT
     mz-bud17 TTCAAATATT TAAATAATAA ATTTTTTAAA AATTATTTTA TGACAACAAT
 mz-spindle18 ATTAAATTTT TCAATAATAA A..TCCAAAA AATTATTTTA AGACAACAAT
 mm-spindle28 ATTAAATTTT TCAATAATAA A..TCCAAAA AATTATTTTA AGACAACAAT
 mj-spindle58 ATTAAATTTT TCAATAATAA A..TCCAAAA AATTATTTTA AGACAACAAT
 mp-spindle68 ATTAAATTTT TTAATAATAA A..TTTAAAA AATTATTTTA AGACAACAAT
 Asphondylia_ .aaaaataaa atttattaat tttttattac aaaattaatt aaacaacaat
 Asphondylia_ .ataaaataa ttattaataa attttattat aaaattaatt aaacaacaat
 Asphondylia_ ....aatata atatttatta atattttact aaaatcaatt aaacaacaat
     mm-bulb25 ..AAAAAATA AAATTAATTT TTTATAAAAT AAAATTAATT AAACAACAAT
     mj-bulb55 .AAAAAAATA AAATTAATTT TTTATAAAAT AAAATTAATT AAACAACAAT
     mz-bulb15 AAAANAAANA AANTAATTTT TTNAAAAANA AAANTNATTN AACCNCCCNT
    mjk-mice41 .AAAAAAANA NTANTAANAN TTTTTT...N AAANTNATTN AACCACCATN
     mt-mice31 .AAAAAAAAA ATTATAAAAA TTTTTT...T AAAATTANTT AACCACCANT
     mj-mice51 .AAAAAAAAT ATTATTAATA TTTTTT...T AAAATTAATT AAACAACAAT
     mz-mice11 ..AAAAAAAT ACTATTAATA TTTTTT...T AAAATTAATT AAACAACAAT
     mm-mice21 .AAAAAAATA TTATTAATAT TTTTTT...T AAAATTAATT AAACAACAAT
     mj-club54 .AAAAATTTT TAATTAATAT TTT.TTTATT AAAATTAATT AAACAACAAT
     mz-bell12 .AAAAATTTT TAATTAATA. TTT.TTTATT AAAATTAATA AAACAACAAT
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       mt-bell32 .AAAAATATT TAATTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
      mjk-bell42 .AAAAATATT TAAGTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
       mm-bell22 .AAAAATATT TAATTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
       mo-club74 .AAAAAATTT TAATTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
       mt-club34 .AAAAATTTT TAATTAATA. ..T.TTTATT AAAATTAATT AACCAACAAT
     mt-bullet39 .AAAAAAATA AAATTAATTT TTTATT.AAT AAAATCAATT AAACAACAAT
     mj-bullet59 .AAAAAAATA AAATTAATTT TTTATT.AAT AAAATCAATT AAACAACAAT
     mz-bullet19 .AAAAAAATA AAATTAATTT TTTATTAAAT AAAATTAATT AAACAACAAT
     mm-bullet29 .AAAAAAATA AAATTAATTT TTTATTAAAT AAAATTAATT AAACAACAAT
     mp-bullet69 .ATAAAAATA AAATTAATTT TCTATTTAAT AAAATCAATT AAACAACAAT
    mjk-bullet49 .ATAAAAATA AAATTAATTT TCTATTTAAT AAAATCAATT AAACAACAAT
     mo-bullet79 .ACAAAAATA AAATTAATTT TCTATTTAAT AAAATCAATT AAACAACAAT

                 251                                                300
        mt-bud37 ATACAATTTA ATT.AATTTA AATATATTTT ATTGTGTATT ATC.AATTAA
        mz-bud17 ATACAATTTA ATT.AATTTA AATATATTTT ATTGTGTATT ATC.AATTAA
    mz-spindle18 ATACAATTTA ATT.AATATA AATAAATTAT AATGTGGATT ATC.AATTAA
    mm-spindle28 ATACAATTTA ATT.AATATA AATAAATTAT AATGTGGATT ATC.AATTAA
    mj-spindle58 ATACAATTTA ATT.AATATA AATAAATTAT AATGTGGATT ATC.AATTAA
    mp-spindle68 ATACAATTTA ATT.AATATA AATAAATTAT AAGGTGGATT ATC.AATTAA
     Asphondylia_ atataattta a.ttaatata agtttattta aatgtgtatt atcaaattaa
     Asphondylia_ atataatttt a.ttaaatta agtatatttt aatgtgtatt atc.aattaa
     Asphondylia_ atataatttt atttaataaa agtatattat aatgtgtatt atc.aattaa
       mm-bulb25 ATATAATTTT ATTAAA.TTA AATATATTAA ATTGGGGATT ATC.AATTAA
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   mj-bulb55 ATATAATTTT ATTAAA.TTA AATATATTAA ATTGGGGATT ATC.AATTAA
   mz-bulb15 NNNAANTTTT ATNANA.TTA ANANTATAAA NTGGGGTATN ACC.ANTNAA
  mjk-mice41 NNTNANCCTN ANAAANTTNA AAAANNTAAA NTGGGGANTA ACC.ANTNAN
   mt-mice31 NTNTAANCTN AANAAATTNA AANANNTNAN ATGGGGAATN ANC.AATNAA
   mj-mice51 ATATAATCTT AATAAATTTA AATATATTGT ATTGTGTATT ATC.AATTAA
   mz-mice11 ATATAATCTC AATAAATTTA AATATATTAT ATTGTGTGTG ATC.AATTAA
   mm-mice21 ATATAATTTT AATAAATTTA AATATATTAT ATTGGGGATT ATC.AATTAA
   mj-club54 ATATAATTTT AATAAA.TTA AATATATTAT ATTGTGTATT ATC.AATTAA
   mz-bell12 ATATAATTTT AATAAA.TTA AATATATTAT GTTGTGTATT ATC.AATTAA
   mt-bell32 ATATAATTTT AATAAA.TCA AACATATTAT ATTGTGTATT ATC.AATTAA
  mjk-bell42 ATATAATTTT AATAAA.TCA AACATATTAT ATTGTGTATT ATC.AATTAA
   mm-bell22 ATATAATTTT AATAAA.TCA AACATATTAT ATTGTGTATT ATC.AATTAA
   mo-club74 ATATAATTTT AATAAA.TTA AATATATTAT ATTGTGTATT ATC.AATTAA
   mt-club34 ATATAATTTT AATAAA.TTA AATATATTAT ATGGGGTATT ATC.AATTAA
 mt-bullet39 ATATAATTTT A.TAAA.ATA AATATATTAT AACGTGTATT ATC.TATTAA
 mj-bullet59 ATATAATTTT A.TAAA.ATA AATATATTAT AACGTGTATT ATC.TATTAA
 mz-bullet19 ATATAATTTA A.TAAA.ATA AATATATTAT AATGTGTATT ATC.AATTAA
 mm-bullet29 ATATAATTTA A.TAAA.ATA AATATATTAT AATGTGTATT ATC.AATTAA
 mp-bullet69 ATATAATTTA ATTAAA.ATA AGTATATTAT AATGTGTATT ATC.AATTAA
mjk-bullet49 ATATAATTTA ATTAAA.ATA AGTATATTAT AATGTGTATT ATC.AATTAA
 mo-bullet79 ATATAATTTA ATTAAA.ATA AGTATATTAT AATGTGTATT ATC.AATTAA

             301                                               350
   mt-bud37 TTAACAAGAT CCTCTAATTT TAAAAAATAC TGCCAATTTA TTTAATTTTT
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    mz-bud17 TTAACAAGAT CCTCTAATTT TAAAAAATAC TGCCAATTTA TTTAATTTTT
mz-spindle18 TAAACAAAAT CCTTTAATTT TTAAAAATAC TGCCATTTTA TTTAATTTTA
mm-spindle28 TAAACAAAAT CCTTTAATTT TTAAAAATAC TGCCATTTTA TTTAATTTTA
mj-spindle58 TAAACAAAAT CCTCTAATTT TTAAAAATAC TGCCACTTTA TTTAATTTTA
mp-spindle68 TAAACAAAAT CCTTTAATTT TTAAAAATAC TGCCATTTTA TTTAATTTTT
Asphondylia_ ttaacaaaat cctctaattt taaataatac taccaaatta tttaatttt.
Asphondylia_ taaacaaaat cctctaattt taaataatac taccaaatta tttaatttt.
Asphondylia_ taaacaaaat cctctaattt taaataatac caccaaatta tttaattttc
   mm-bulb25 TTAACAAAAT CCCCTAATTT TTAAAAA.TA CTACCAAATT AATTAATTTT
   mj-bulb55 TTAACAAAAT CCTCAAATTT TTAAAAA.TA CTACCAAATT AATTAATTTT
   mz-bulb15 TNACCAAAAC CCCCNANTTT TAAAAANGNN CCNCNAAATN AATTANTTTC
  mjk-mice41 TNACCAAANC CCCCNANTTT TNAAAANNCC NCCCAANTNA NTNANTTTTN
   mt-mice31 TTANCAAANN CCNCTAATTT TNAAAAANNC NNCCAAATNA ATNANTTTTN
   mj-mice51 TTAACAAAAT CCTCTAATTT TTAAGAATAC TACCAAATTA ATTAAGTTTT
   mz-mice11 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA ATTAATTTTT
   mm-mice21 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA ATTAATTTTT
   mj-club54 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTA
   mz-bell12 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTA
   mt-bell32 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTA
  mjk-bell42 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTA
   mm-bell22 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAGATTA TTTAATTTTA
   mo-club74 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTA
   mt-club34 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTA
 mt-bullet39 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTA
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 mj-bullet59 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTA
 mz-bullet19 TTGACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTT
 mm-bullet29 TTGACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTT
 mp-bullet69 TAAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA ATTAATTTTT
mjk-bullet49 TCAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA CTTAATTTTT
 mo-bullet79 TTAACAAAAT CCTCTAATTT TTAAAAATAC TACCAAATTA TTTAATTTTT

             351                                              400
     mt-bud37 TTTAAATTAA AAATTAATTT TTTTAA...A AAAAAAAAGG GGATCTAATC
     mz-bud17 TTTAAATTAA AAATTAATTT TTTTAA...A AAAAAAAAGG GTATCTAAAC
 mz-spindle18 TTATAAT.TT TTACTAATTA TTTTTA...A TCTTAAAATT AAAATAATAG
 mm-spindle28 TTATAAT.TT TTACTAATTA TTTTTA...A TCTTAAAATT AAAATAATAG
 mj-spindle58 ATCAATC.TA CTACTAATTA TTTATT...A TCATTAAAAT AATAGGGTAG
 mp-spindle68 ATATATTATT TTACTAATTA TTTATT...A AAATTAAAAT AATAGGGTAT
 Asphondylia_ ..taaaaata aaaatttact aattaaaaa. aaaattaatc cttataata~
 Asphondylia_ ..ttttaatt taataataat tattaaaaat ttaataaatt tatataata~
 Asphondylia_ aaaaaaaatt taatattact tattaaaata ttattaaact aatataata~
     mm-bulb25 CTCTTTTTAT AGA.ATAATA GGCCAA..TA AAT.TATATT ATTATAATAG
     mj-bulb55 CTCTTTTAAT AGA.ATAATA ATTAAT..AA ACTCTATATT ATTATAATAG
     mz-bulb15 CNCTTTTAAN AAA.ATANNA ATTAA....N AANTCNTATT ATNANAGNGG
    mjk-mice41 AAAANANTTT TNA.ANANTN ANAN.....C CCTNAAAAAA TTNNNANAGG
     mt-mice31 AANAANATTT TNA.AAAATT AANA.....N CCNTAAAAAA NTTATANNNG
     mj-mice51 AATAATATTT TTA.ATAATT AATA.....T CCATAAAAAA ATTATAATAG
     mz-mice11 AATAATATTT TTA.ATAATT AATA.....T CCATAAAAAA ATTATAATAG
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mm-mice21    AATATCATAT TTA.ATAATT AGTA.....T TCATAAAAAA ATTATAATAG
mj-club54    AAATAAAAAA TTA.ATAATT AAATAT...A TAATAAAAAA ATTATAATAG
mz-bell12    AAATAAAAAA TTA.ATAATT AAATATATAA TAATAAAAAA ATTAGAATAG
mt-bell32    AAATAAAAAA ATT.AATAAT TAAATT...T ATATATAAAA ATTATAATAG
mjk-bell42   AAATAAAAAA ATT.AATAAT TAAATT...T ATATATAAAA ATTATAATAG
mm-bell22    AAATAAAAAA ATT.AATAAT TAAATT...T ATATATAAAA ATTATAATAG
mo-club74    AAATAAAAAT TTA.ATAATT AAATAT...A TAATAAAAAA ATTATAATAG
mt-club34    AAATAAAAAT TTA.ATAATT AAATAT...A TAATAAAAAA ATTATAATAG
mt-bullet39  ATTTTTTAAA TTA.ATAATT AATA.....C AAATAAAAAA ATTATAATAG
mj-bullet59  ATTTTTTAAA TTA.ATAATT AATA......  GAATAAAAAA ATTATAATAG
mz-bullet19  A.TTTTAATA TTA.ATAATT AATA.....A AA..AAAAAT ATTATAATAG
mm-bullet29  A.TTTTAATA TTA.ATAATT AATA.....A AA..AAAAAT ATTATAATAG
mp-bullet69  A.TTTTAAAA TTA.ATAATT AATA.....T AATTAAAAAA ATTATAATAG
mjk-bullet49 A.CTTTAAAA TTA.ATAATT AATA.....T AATTAAAAAA ATTATAATAG
mo-bullet79  A.TTTTAAAA TTA.ATAATT AATA.....T AATTAAAAAA ATTATAATAG

             401                    423
mt-bud37     CTAGTTTAGT CA~~~~~~~~ ~~~
mz-bud17     CTAATTTA~~ ~~~~~~~~~~ ~~~
mz-spindle18 GGTATCTAAT CCCTAGTTTA ~~~
mm-spindle28 GGTATCTAAT CCTAGTTTA~ ~~~
mj-spindle58 GGAATCCTAG CCTA~~~~~~ ~~~
mp-spindle68 CTAATCCTAG TTTA~~~~~~ ~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~
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Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~
  mm-bulb25 GGCATCTAAT CCTAGTTTA~ ~~~
  mj-bulb55 GGGATCTAAT CCTAGTTTAA ~~~
  mz-bulb15 GGNACCNANC CCNNNNTAAN ~~~
 mjk-mice41 GGANCCNANC CCAGGTTAA~ ~~~
  mt-mice31 GGNANCTAAC CCNNGTTAA~ ~~~
  mj-mice51 GGGATCTAAT CCTAGTTTA~ ~~~
  mz-mice11 GGGGGCTAAT CCTAGTTTAT ATA
  mm-mice21 GGGATCTAAT CCTAGTTTA~ ~~~
  mj-club54 GGTATCTAAT CCTAGTTTA~ ~~~
  mz-bell12 GGTATCTAAT CCAAGTTTA~ ~~~
  mt-bell32 GGTATCTAAT CCTAGTTTA~ ~~~
 mjk-bell42 GGTATCTAAT CCTAGTTTA~ ~~~
  mm-bell22 GGTATCTAAT CCTAGTTTA~ ~~~
  mo-club74 GGTATCTAAT CCTAGTTTA~ ~~~
  mt-club34 GGTATCTAAC CCTAGTTTA~ ~~~
 mt-bullet39 GGTATCTAAT CCTAGTTTAA ~~~
 mj-bullet59 GGTATCTAAT CCTAGTTTAA ~~~
 mz-bullet19 GGTATCTAAT CCTAGTTTAA ~~~
 mm-bullet29 GGTATCTAAT CCTAGTTTAA ~~~
 mp-bullet69 GGTATCTAAT CCAAGTTTAA ~~~
mjk-bullet49 GGTATCTAAT CCTAGTTTAA ~~~
 mo-bullet79 GGTATCTAAT CCTAGTTTAA ~~~
```

Appendix 6. COI Gene UPGMA Genetic Distances

Generic Distances

Calculated over: 44 to 443
Considering all base positions
Correction method: Kimura 2-parameter
Distances are: estimated number of substitutions per 100 bases
Symmatrix version 1
Number of matrices: 1

//
Matrix 1, dimension: 40

Key for column and row indices:

1 Drosophila_bifasciata_COI
2 Drosophila_ambigua_COI
3 Asphondylia_sphaera-Asphodyliini
4 Asphondylia_gennadii-Asphodyliini
5 Asphondylia_itoi-Asphodyliini
6 mj-blister56
7 mo-blister76
8 mt-blister36
9 mjk-bulb45
10 mj-bulb55

11 mm-bulb25
12 mt-bullet39
13 mj-bullet59
14 mz-bullet19
15 mm-bullet29
16 mo-bullet79
17 mjk-bullet49
18 mp-bullet69
19 mj-mice51
20 mjk-mice41
21 mt-mice31
22 mz-mice11
23 mm-mice21
24 mt-bell32
25 mm-bell22
26 mjk-bell42
27 mt-club34
28 mo-club74
29 mp-club64
30 mk-bird83
31 mz-bell12
32 mj-club54
33 mjk-club44
34 mp-spindle68

35 mt-spindle38
36 mm-spindle28
37 mz-spindle18
38 mj-spindle58
39 mz-bud17
40 mt-bud37

Matrix 1: Part 1

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.00	8.48	130.29	134.62	149.62	133.96	132.71	146.44	139.37	135.66	144.60	125.71
2		0.00	141.58	141.88	149.62	146.23	138.87	148.95	151.97	147.49	155.94	134.36
3			0.00	12.94	16.02	31.94	35.96	34.82	25.29	25.64	29.73	25.64
4				0.00	14.13	28.91	32.33	33.62	23.54	23.20	28.19	23.89
5					0.00	30.79	33.90	31.29	24.60	25.65	26.41	23.90
6						0.00	22.50	19.51	23.81	22.10	27.01	22.86
7							0.00	27.95	25.57	25.22	27.72	29.66
8								0.00	27.08	26.73	30.44	30.52
9									0.00	2.83	9.43	18.53
10										0.00	9.73	19.50
11											0.00	24.26
12												0.00
13												

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Matrix 1: Part 2

	13	14	15	16	17	18	19	20	21	22	23	24
1	131.44	130.72	129.49	125.59	127.11	124.45	126.15	126.15	126.15	124.96	120.77	120.47
2	137.99	127.64	125.25	126.15	125.25	122.66	130.41	130.41	130.41	129.16	127.15	131.08
3	26.73	23.90	23.20	21.51	21.50	20.83	22.22	22.22	22.22	22.57	20.51	21.49
4	27.08	23.54	22.85	20.49	21.16	21.16	19.18	19.18	19.18	19.51	19.50	19.50
5	23.88	24.23	23.54	21.16	21.16	21.16	22.51	22.51	22.51	22.85	20.49	21.17
6	27.08	24.93	24.58	22.52	22.51	23.20	23.88	23.88	23.88	23.88	22.51	20.49
7	32.33	28.55	29.31	28.17	27.81	28.17	27.08	27.08	27.08	27.08	27.82	28.17
8	30.20	29.07	28.31	26.09	26.10	26.09	27.17	27.17	27.17	27.17	26.80	24.66
9	20.16	16.62	16.62	15.06	15.68	15.36	17.24	17.24	17.24	17.24	16.93	14.13
10	20.83	17.88	17.88	15.99	16.61	16.30	17.24	17.24	17.24	17.24	17.24	15.36
11	24.58	20.85	20.85	18.20	18.85	18.52	20.84	20.84	20.84	20.84	20.50	18.86
12	6.60	11.44	11.15	11.73	11.44	11.44	12.03	12.03	12.03	12.33	11.15	10.27
13	0.00	14.44	14.13	14.13	13.83	13.83	13.24	13.24	13.24	13.54	12.63	12.03
14		0.00	0.76	6.65	6.36	6.08	11.82	11.82	11.82	12.12	10.90	11.17
15			0.00	6.08	5.80	5.53	11.82	11.82	11.82	12.12	10.90	10.87
16				0.00	0.76	0.76	9.13	9.13	9.13	9.42	8.84	8.27

17	0.00	0.51	9.71	9.71	9.71	10.00	9.41	9.12
18		0.00	9.42	9.42	9.42	9.71	9.13	8.55
19			0.00	-0.00	-0.00	0.25	2.58	6.60
20				0.00	-0.00	0.25	2.58	6.60
21					0.00	0.25	2.58	6.60
22						0.00	2.84	6.88
23							0.00	5.23
24								0.00
25								
26								
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Matrix 1: Part 3

	25	26	27	28	29	30	31	32	33	34	35	36
1	120.47	120.47	118.36	118.36	115.67	120.16	118.80	114.67	113.97	145.67	144.15	136.71
2	131.08	131.08	129.86	129.86	125.59	127.11	126.75	124.45	123.62	145.12	143.69	145.72
3	21.49	21.49	20.16	20.16	20.49	20.82	20.82	19.83	20.82	23.62	23.62	25.37
4	19.50	19.50	18.21	18.21	18.53	18.85	18.85	18.53	18.85	22.57	22.57	23.61
5	21.17	21.17	20.50	20.50	20.84	21.16	20.84	20.84	21.16	23.96	23.96	23.61
6	20.49	20.49	20.49	20.49	20.16	20.16	20.82	20.49	19.45	21.26	20.91	24.67
7	28.17	28.17	29.66	29.66	28.91	28.55	29.66	28.54	26.99	26.15	26.15	24.66
8	24.66	24.66	25.01	25.01	25.02	25.02	24.67	25.02	25.65	28.04	27.67	31.43
9	14.13	14.13	15.36	15.36	15.36	15.05	15.67	15.36	15.32	20.91	20.91	23.62
10	15.36	15.36	16.61	16.61	16.61	16.30	16.93	16.61	16.56	21.24	21.24	23.96
11	18.86	18.86	20.52	20.52	20.17	20.51	20.51	20.84	20.79	25.36	25.36	25.72
12	10.27	10.27	10.56	10.56	10.56	9.40	9.98	10.56	11.15	22.96	22.63	22.95
13	12.03	12.03	12.33	12.33	12.33	11.44	11.73	12.33	12.63	24.66	24.31	25.72
14	11.17	11.17	10.57	10.57	10.87	9.70	11.17	10.87	11.46	21.23	20.90	23.30
15	10.87	10.87	10.28	10.28	10.57	9.41	10.87	10.57	11.16	20.57	20.24	22.96
16	8.27	8.27	7.99	7.99	8.84	8.27	8.55	8.84	9.41	20.89	20.56	22.60
17	9.12	9.12	8.55	8.55	9.41	8.84	9.12	9.41	9.98	20.23	19.90	21.59
18	8.55	8.55	7.99	7.99	8.84	8.27	8.55	8.84	9.41	19.91	19.58	22.27
19	6.60	6.60	7.17	7.17	7.17	6.89	6.89	7.17	7.73	21.22	20.89	23.61

20	6.60	6.60	7.17	7.17	7.17	6.89	6.89	7.17	7.73	21.22	20.89	23.61
21	6.60	6.60	7.17	7.17	7.17	6.89	6.89	7.17	7.73	21.22	20.89	23.61
22	6.88	6.88	7.45	7.45	7.45	7.18	7.17	7.45	8.01	21.56	21.22	23.96
23	5.23	5.23	5.78	5.78	5.78	5.51	5.50	5.78	6.33	19.24	18.92	22.92
24	-0.00	-0.00	3.10	3.10	3.10	3.10	2.31	3.10	3.62	18.26	17.94	21.56
25	0.00	-0.00	3.10	3.10	3.10	3.10	2.31	3.10	3.62	18.26	17.94	21.56
26		0.00	3.10	3.10	3.10	3.10	2.31	3.10	3.62	18.26	17.94	21.56
27			0.00	-0.00	1.79	1.80	2.58	1.79	2.57	19.57	19.24	22.23
28				0.00	1.79	1.80	2.58	1.79	2.57	19.57	19.24	22.23
29					0.00	2.06	1.80	0.51	1.53	19.57	19.24	22.23
30						0.00	2.32	2.06	3.10	19.24	18.92	20.89
31							0.00	1.80	2.84	19.89	19.57	22.57
32								0.00	1.02	19.89	19.57	21.90
33									0.00	19.89	19.56	21.89
34										0.00	0.25	11.86
35											0.00	11.55
36												0.00
37												
38												
39												
40												

Matrix 1: Part 4

	37	38	39	40
1	140.37	142.03	147.43	126.96
2	150.03	144.53	147.43	137.67
3	25.72	26.37	22.17	26.11
4	23.96	24.23	21.84	25.40
5	23.96	24.23	22.17	27.58
6	25.02	23.91	23.20	23.60
7	25.02	24.23	25.36	29.38
8	31.79	29.05	33.24	27.60
9	23.98	23.55	23.57	23.55
10	24.32	23.89	25.32	24.95
11	26.08	25.29	26.72	27.51
12	23.31	22.88	21.56	26.80
13	26.08	24.93	21.85	30.51
14	23.66	22.18	22.87	25.74
15	23.32	21.85	22.54	25.37
16	22.96	20.85	21.84	22.58
17	21.95	19.87	21.17	22.94
18	22.63	20.53	21.84	22.58
19	23.96	21.49	21.83	25.73
20	23.96	21.49	21.83	25.73
21	23.96	21.49	21.83	25.73
22	24.31	21.83	22.17	26.09

23	23.27	20.82	21.16	24.66
24	21.89	20.16	22.21	22.23
25	21.89	20.16	22.21	22.23
26	21.89	20.16	22.21	22.23
27	22.58	21.83	20.85	22.92
28	22.58	21.83	20.85	22.92
29	22.58	21.49	21.20	23.26
30	21.23	20.16	20.22	23.61
31	22.92	21.16	21.53	22.92
32	22.24	21.16	21.53	22.57
33	22.24	21.16	21.17	22.85
34	11.84	9.78	19.23	25.47
35	11.54	9.48	19.23	25.47
36	0.51	2.05	20.55	26.52
37	0.00	2.05	20.55	26.16
38		0.00	21.16	26.08
39			0.00	26.54
40				0.00

#NEXUS

```
begin trees;
tree Tree_1 = (('Drosophila_bifasciata_COI':4.24,'Drosophila_ambigua_COI':4.24)
:65.99,((((((('Asphondylia_sphaera-Asphodyliini':6.47,'Asphondylia_gennadii-Asphodyliin'
:6.47):1.07,'Asphondylia_itoi-Asphodyliini':7.54):3.70,((('mp-spindle68'
:0.12,'mt-spindle38':0.12):5.38,(('mm-spindle28':0.25,'mz-spindle18'
:0.25):0.77,'mj-spindle58':1.02):4.48):4.30,'mz-bud17':9.81)
:1.43):1.28,((('mjk-bulb45':1.41,'mj-bulb55':1.41):3.38,
'mm-bulb25':4.79):5.91,(('mt-bullet39':3.30,'mj-bullet59':3.30)
:2.87,((('mz-bullet19':0.38,'mm-bullet29':0.38):2.70,('mo-bullet79'
:0.38,('mjk-bullet49':0.25,'mp-bullet69':0.25):0.12):2.70)
:1.68,((((('mj-mice51':-0.00,'mjk-mice41':-0.00):0.00,'mt-mice31'
:-0.00):0.12,'mz-mice11':0.12):1.20,'mm-mice21':1.32):1.71,
(((('mt-bell32':-0.00,'mm-bell22':-0.00):0.00,'mjk-bell42':-0.00)
:1.15,'mz-bell12':1.15):0.28,((('mt-club34':-0.00,'mo-club74'
:-0.00):0.90,'mk-bird83':0.90):0.30,(('mp-club64':0.25,'mj-club54'
:0.25):0.45,'mjk-club44':0.70):0.50):0.24):1.59):1.72)
:1.42):4.52):1.81):0.72,'mt-bud37':13.23):1.53,(('mj-blister56'
:9.76,'mt-blister36':9.76):2.86,'mo-blister76':12.61):2.15)
:55.47):0.00;
endblock;
```

```
 Symbol comparison table: pileupdna.cmp  CompCheck: 6876

                  GapWeight: 5
            GapLengthWeight: 1

PileUp  MSF: 539  Type: N  March 17, 2008 00:18  Check: 5765 ..

 Name: Drosophila_bifasciata_COI  Len:   539  Check: 6505  Weight:  1.00
 Name: Drosophila_ambigua_COI  Len:   539  Check: 3485  Weight:  1.00
 Name: Asphondylia_sphaera-Asphodyliini  Len:   539  Check: 3151  Weight:  1.00
 Name: Asphondylia_gennadii-Asphodyliini  Len:   539  Check:  785  Weight:  1.00
 Name: Asphondylia_itoi-Asphodyliini  Len:   539  Check: 6174  Weight:  1.00
 Name: mj-blister56     Len:   539  Check:  814  Weight:  1.00
 Name: mo-blister76     Len:   539  Check: 7103  Weight:  1.00
 Name: mt-blister36     Len:   539  Check: 7532  Weight:  1.00
 Name: mjk-bulb45       Len:   539  Check: 9081  Weight:  1.00
 Name: mj-bulb55        Len:   539  Check: 7176  Weight:  1.00
 Name: mm-bulb25        Len:   539  Check: 8203  Weight:  1.00
 Name: mt-bullet39      Len:   539  Check: 7274  Weight:  1.00
 Name: mj-bullet59      Len:   539  Check: 8315  Weight:  1.00
 Name: mz-bullet19      Len:   539  Check: 9452  Weight:  1.00
 Name: mm-bullet29      Len:   539  Check: 8211  Weight:  1.00
 Name: mo-bullet79      Len:   539  Check: 8238  Weight:  1.00
```

Name:	mjk-bullet49	Len:	539	Check:	61	Weight:	1.00
Name:	mp-bullet69	Len:	539	Check:	597	Weight:	1.00
Name:	mj-mice51	Len:	539	Check:	1146	Weight:	1.00
Name:	mjk-mice41	Len:	539	Check:	5056	Weight:	1.00
Name:	mt-mice31	Len:	539	Check:	9984	Weight:	1.00
Name:	mz-mice11	Len:	539	Check:	4270	Weight:	1.00
Name:	mm-mice21	Len:	539	Check:	1313	Weight:	1.00
Name:	mt-bell32	Len:	539	Check:	8990	Weight:	1.00
Name:	mm-bell22	Len:	539	Check:	9051	Weight:	1.00
Name:	mjk-bell42	Len:	539	Check:	7672	Weight:	1.00
Name:	mt-club34	Len:	539	Check:	8252	Weight:	1.00
Name:	mo-club74	Len:	539	Check:	7638	Weight:	1.00
Name:	mp-club64	Len:	539	Check:	1580	Weight:	1.00
Name:	mk-bird83	Len:	539	Check:	1076	Weight:	1.00
Name:	mz-bell12	Len:	539	Check:	76	Weight:	1.00
Name:	mj-club54	Len:	539	Check:	2071	Weight:	1.00
Name:	mjk-club44	Len:	539	Check:	9331	Weight:	1.00
Name:	mp-spindle68	Len:	539	Check:	6218	Weight:	1.00
Name:	mt-spindle38	Len:	539	Check:	2369	Weight:	1.00
Name:	mm-spindle28	Len:	539	Check:	2379	Weight:	1.00
Name:	mz-spindle18	Len:	539	Check:	9057	Weight:	1.00
Name:	mj-spindle58	Len:	539	Check:	8359	Weight:	1.00
Name:	mz-bud17	Len:	539	Check:	3867	Weight:	1.00
Name:	mt-bud37	Len:	539	Check:	3853	Weight:	1.00

```
//
                1                                                 50
Drosophila_b ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~ccaacantt attttgattt
Drosophila_a ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~ttat accaacattt attctgattc
Asphondylia_ tcgtttaaat aatataagat tttgactatt acctccatca ttaactattt
Asphondylia_ tcgccttaat aatataagat tttgactttt acctccatca ttaacaattt
Asphondylia_ tcgattaaat aatataagat tttgacttct tcctccatca ttaactatcc
mj-blister56 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~TCAATTAT
mo-blister76 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GTGT GATCAAAACA
mt-blister36 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~CTTAAG
   mjk-bulb45 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~C AAAACATATA
    mj-bulb55 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~CAT TCTACACTAT
    mm-bulb25 ~~~~~TTACA TAAATTATAT TTCCCCAGGG TATGTTTTTT TTTATTTTTA
  mt-bullet39 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~CTATT AAGACCATCA CTTTTAATAA
  mj-bullet59 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~G GGTTATTTTT TTGTTTCACC
  mz-bullet19 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~CT ACCACCATCA ATTCTAATTA
  mm-bullet29 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGGCG ACCACCATCA ATTCTAATTA
  mo-bullet79 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~CTTATT ACCTCCATCA ATTTTAATTC
mjk-bullet49 ~~~~~~~~~~ ~~~~~~TTTA GTTTCGGGTT TCCTCCATCA ATTTTAATTC
  mp-bullet69 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~GG TTCCTCCTCA ATTTTAATTC
    mj-mice51 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~ATT ACCTCCTTCA CTTTCTATTC
   mjk-mice41 ~~~~~~~~~~ ~~~~~~~~~~ ~~~GATTATT ACCTCCTTCA CTTTCTATTC
    mt-mice31 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~TATT ACCTCCTTCA CTTTCTATTC
```

```
   mz-micell ~~~~~~~~~~ ~~~~~~TTAG ATCCGAGAGG ACCTCCTTCA CTTTCTATTC
   mm-mice21 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~CTTT ACCTCCATCA CTTTCTATTC
   mt-bell32 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~TTACT ACCTCCATCA TTATCTATTC
   mm-bell22 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GGCT ACCTCCATCA TTATCTATTC
  mjk-bell42 ~~~~~~~~~~ ~~~~~~~TAG ATTTGATTCT ACCTCCATCA TTATCTATTC
   mt-club34 ~~~~~~~~~~ ~~~TATTAAT TTTCCGGGGA ACCTCCATCA TTATCTATTC
   mo-club74 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ACCTCCATCA TTATCTATTC
   mp-club64 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ACCTCCTTCA TTGTCTATTC
   mk-bird83 ~~~~~~~~~~ ~~~~~~~~~T ACACGGGGCT ACCTCCATCA CTATCTATTC
   mz-bell12 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GGAT ACCTCCATCA CTATCTATTC
   mj-club54 ~~~~GGCTAT TTGTAGTATT ATCGAGGACT ACCTCCTTCA TTATCTATTC
  mjk-club44 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~T TTATCTATTC
mp-spindle68 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~CCCCCTCT TTACCTTATT
mt-spindle38 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~CTT TAACCTTATT
mm-spindle28 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~AA AAATTTTATT
mz-spindle18 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~AATTTTATT
mj-spindle58 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~TT TAATTTTATT
    mz-bud17 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~TTC AAACTTTATA
    mt-bud37 ~~~~~~~~~~ ~~~~TTTTAA ATACGGGTCT CCCTCCTTCT TTAATATTAC

             51                                               100
Drosophila_b tttggccacc cagaagttta tattttaatt ttaccaggat ttggaataat
Drosophila_a tttggtcacc cagaagttta cattttaatt ttaccaggat ttgggataat
Asphondylia_ tattaataag aagaattatt .gaaaacggg actggaaccg gatgaactat
```

```
Asphondylia_ tattaataag aagaattatt .gaaagaggg acaggaacag gatgaacagt
Asphondylia_ tattaataag ttcaattatc .gaaagaggg acaggaacag gctgaacaat
mj-blister56 CTAATTAAGA ATGATTAATA AAAACTGGGA CTGGGGACTG GATGAACAGT
mo-blister76 TTAATTAAGA AGAATAATAG AAACTTGGAA CTAGGAACTG GATGAACAGT
mt-blister36 GCATTTGAAG GGCAGCACAA CAGGCGAATC AGGAACATGG TTCGAACAGT
   mjk-bulb45 TTATATAAAG AAAAACTGGT AAAAACAGGA ACAGGAACGG GATGAACTGT
    mj-bulb55 GTTATTAAAG AAAAACTTGT AAAAACAGGA ACAGGAACGG GATGAACTGT
    mm-bulb25 CCCGGGGATT TGAATTCTGT AGAAACAGGA ACAGGGACAG GATGAACTGT
  mt-bullet39 TTATTTTAAG AATAATAATT .GAAATAGGA ACTGGAACAG GATGAACTAT
  mj-bullet59 GGGTTTTAAG AATAATAGGG .GAAAGAGGA GCTGGAACAG GATGAACTAT
  mz-bullet19 TTCTTTTAAG AAGAATAATT .GAAAGAGGA ACTGGAACAG GATGAACTAT
  mm-bullet29 TTCTTTTAAG AAGAATAATT .GAAAGAGGA ACTGGAACAG GATGAACTAT
  mo-bullet79 TTCTTATAAG AAGAATAATT .GAAAAAGGA ACTGGAACAG GATGAACTGT
 mjk-bullet49 TTCTTATAAG AAGAATAATT .GAAAAAGGA ACTGGAACAG GATGAACTAT
  mp-bullet69 TTCTTATAAG AAGAATAATT .GAAAAAGGA ACTGGAACAG GATGAACTAT
    mj-mice51 TTCTTTTAAG AAGAATAATT .GAAAGGGGA ACTGGAACAG GATGAACTGT
   mjk-mice41 TTCTTTTAAG AAGAATAATT .GAAAGGGGA ACTGGAACAG GATGAACTGT
    mt-mice31 TTCTTTTAAG AAGAATAATT .GAAAGGGGA ACTGGAACAG GATGAACTGT
    mz-mice11 TTCTTTCAAG AAGAATAATT .GAAAGGGGA ACTGGAACAG GATGAACTGT
    mm-mice21 TTCTTTTAAG AAGAATAATT .GAAAGAGGA ACTGGAACAG GATGAACAGT
    mt-bell32 TTATTTTAAG AAGAATAATT .GAAAGAGGA ACTGGAACAG GATGAACAGT
    mm-bell22 TTATTTTAAG AAGAATAATT .GAAAGAGGA ACTGGAACAG GATGAACAGT
   mjk-bell42 TTATTTTAAG AAGAATAATT .GAAAGAGGA ACTGGAACAG GATGAACAGT
    mt-club34 TTATTATAAG AAGAATAATT .GAAAGAGGC ACTGGAACAG GATGAACAGT
```

```
     mo-club74 TTATTATAAG AAGAATAATT .GAAAGAGGC ACTGGAACAG GATGAACAGT
     mp-club64 TTATTTTAAG AAGAATAATT .GAAAGAGGT ACTGGAACAG GATGAACAGT
     mk-bird83 TTATTTTAAG AAGAATAATT .GAAAGAGGT ACTGGAACAG GATGAACAGT
     mz-bell12 TTATTTTAAG AAGAATAATT .GAAAGAGGT ACTGGAACAG GATGAACAGT
     mj-club54 TTATTTTAAG AAGAATAATT .GAAAGAGGT ACTGGAACAG GATGAACAGT
    mjk-club44 TTATTTTAAG AAGAATAATT AGAAAGAGGA CTAGGAACAG GATGAACAGT
   mp-spindle68 ATTAATT.AG AAGAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
   mt-spindle38 ATTAATT.AG AAGAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
   mm-spindle28 ATTAA.TAAG AATAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
   mz-spindle18 ATTAA.TCAG AATAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
   mj-spindle58 ATTAATTCAG AATAATTATT .GAAAATGGA ACAGGAACAG GATGAACAAT
       mz-bud17 TATTAATTAA AAGAATAGTA .GAAACTGGA TCAGGAACAG GATGAACTAT
       mt-bud37 TTTTAATCAG AAGAAT.GGT AGAAACAGGG ACAGGGACAG GATGAACTGT

                101                                              150
   Drosophila_b ttctcatatt attagtcaag aatcagggaa aaaggaaact tttggatctt
   Drosophila_a ttcacatatt attagtcaag aatcaggaaa gaaggaaact tttggatctt
   Asphondylia_ ttatcctcct ttatcatcaa ttattgctca taatggaaga tcaactgatt
   Asphondylia_ ttatcccccct ttatcctcaa ttattgctca taatagaaga tcaacagatt
   Asphondylia_ ttacccccca ttatcatcta ttattgctca taatagaaga tcaactgatt
   mj-blister56 TTATCCCCCT CTTTCATCAA CAATTGCTCA TACTGGATCT TCAGTATATT
   mo-blister76 CTATCCACCC CTTTCTTCTA TTATTGCACA TACAGGCTCT TCTGTAGATT
   mt-blister36 TTACCCTCCA CTTTCATCAA CTATTGCTCA TACAGGATCA TCTGTTGATT
      mjk-bulb45 ATATCCACCA CTTTCATCAA TTATTGCCCA TAATGGAGCA TCTGTTGACT
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mj-bulb55	ATATCCACCA	CTTTCATCAA	TTATTGCCCA	TAATGGAGCA	TCTGTTGACT
mm-bulb25	ATACCCACCA	CTCTCATCAA	TTATTGCCCA	TAATGGTGCG	TCTGTTGACT
mt-bullet39	TTATCCCCCT	CTTTCTTCAA	TTATAGCACA	TAGTAGAGCA	TCTGTAGATT
mj-bullet59	TTACCCCCCT	CTTTCTTCAA	TTATAGCACA	TAAGGGAGCA	TCTGTAGACT
mz-bullet19	TTATCCACCT	CTTTCCGCAA	TTATTGCACA	TAATAGACCA	TCTGTTGATC
mm-bullet29	TTATCCGCCT	CTTTCCGCAA	TTATTGCACA	TAATAGACCA	TCTGTTGATC
mo-bullet79	TTACCCCCCT	CTATCTTCAA	TTATTGCGCA	TAATGGACCA	TCTGTTGATC
mjk-bullet49	TTACCCCCCT	CTATCTTCAA	TTATTGCACA	TAATGGACCA	TCTGTTGATC
mp-bullet69	TTACCCTCCT	CTATCTTCAA	TTATTGCACA	TAATGGACCA	TCTGTTGATC
mj-mice51	CTACCCACCT	CTTTCTTCTA	TTATAGCCCA	TAATAGATCA	TCAGTAGATT
mjk-mice41	CTACCCACCT	CTTTCTTCTA	TTATAGCCCA	TAATAGATCA	TCAGTAGATT
mt-mice31	CTACCCACCT	CTTTCTTCTA	TTATAGCCCA	TAATAGATCA	TCAGTAGATT
mz-mice11	CTACCCACCT	CTTTCTTCTA	TTATAGCCCA	TAATAGATCA	TCAGTAGATT
mm-mice21	CTACCCACCT	CTTTCTTCTA	TTATAGCCCA	TAATAGATCA	TCAGTAGATT
mt-bell32	ATACCCTCCT	CTTTCTTCAA	TTATAGCGCA	TAATGGATCA	TCAGTAGATT
mm-bell22	ATACCCTCCT	CTTTCTTCAA	TTATAGCGCA	TAATGGATCA	TCAGTAGATT
mjk-bell42	ATACCCTCCT	CTTTCTTCAA	TTATAGCGCA	TAATGGATCA	TCAGTAGATT
mt-club34	TTATCCTCCT	CTTTCTTCAA	TTATAGCTCA	TAATGGATCA	TCAGTAGATT
mo-club74	TTATCCTCCT	CTTTCTTCAA	TTATAGCTCA	TAATGGATCA	TCAGTAGATT
mp-club64	TTATCCTCCT	CTTTCTTCAA	TTATAGCTCA	TAATGGATCG	TCAGTAGATT
mk-bird83	TTATCCTCCT	CTTTCTTCAA	TTATAGCTCA	TAATGGATCA	TCAGTAGATT
mz-bell12	TTACCCTCCT	CTTTCTTCAA	TTATAGCTCA	TAATGGATCA	TCAGTAGATT
mj-club54	TTATCCTCCT	CTTTCTTCAA	TTATAGCTCA	TAATGGATCA	TCAGTAGATT
mjk-club44	TTATCCTCCT	CTTTCTTCAA	TTATAGCTCA	TAATGGATCA	TCAGTAGATT

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mp-spindle68 TTACCCTCCT CTTTCTTCAA TTATTGCACA TACTAGACCT TCAGTAGACT
mt-spindle38 TTACCCTCCT CTTTCTTCAA TTATTGCACA TACTAGACCT TCAGTAGACT
mm-spindle28 TTATCCCCCC CTTTCTTCTA TTACTGCACA TACTAGAAAT TCAGTAGATT
mz-spindle18 TTATCCCCCC CTTTCTTCTA TTAATGCACA TACTAGAAAT TCAGTAGATT
mj-spindle58 TTACCCCCCC CTTTCTTCTA TTACTGCACA TACTAGAACT TCAGTAGATT
    mz-bud17 TTATCCCCCC TTATCTTCCA TTATTGCTCA TACAAGTTCT TCAGTAGATT
    mt-bud37 ATATCCTCCT CTTTCTTCAT CAATTGCCCA TACTGGCTCA TCAGTTGATT

             151                                              200
Drosophila_b taggaataat ttatgctata cttgcaattg gattattagg atttattgta
Drosophila_a taggaataat ttatgcaata cttgctattg gattactatg atttattgta
Asphondylia_ tatcaatctt tt...cactt catatcgcag gaatttcttc tattttagga
Asphondylia_ tatctatttt tt...cactt catattgctg gtatctcttc tattttagga
Asphondylia_ tatcaatttt tt...catta cacattgcag gaatctcttc tattttaggg
mj-blister56 TTTCTATTTT TT...CTCTT CATATTGCTG GAATTTCTTC TATTTTAGGA
mo-blister76 TTTCAATTTT TT...CACTA CATATCGCGG GAATCTCATC CATTTTGGGG
mt-blister36 TTTCTATTTT TT...CACTA CATATTGCAG GAATTTCATC AATCCTAGGA
   mjk-bulb45 TATCATTTTT TT...CTCTT CATATTGCAG GAATTTCATC ATTTTTAGGA
    mj-bulb55 TATCATTTTT TT...CTCTT CATATTGCAG GAATTTCATC ATTTTTAGGA
    mm-bulb25 TATCAATTTT TT...CTCTC CATATTGCAG GAATCTCATC AATTTTGGGA
  mt-bullet39 TATCTATTTT TT...CACTT CATATAGCAG GAATTTCATC AATTTTAAGA
  mj-bullet59 TATCTATTTT TT...CACTT CATATAGCAG GAATTTCATC AATTTTAAGA
  mz-bullet19 TATCTATTTT TT...CACTT CATATTGCAG GAATTTCATC AATTTTAGGA
  mm-bullet29 TATCTATTTT TT...CACTT CATATTGCAG GAATTTCATC AATTTTAGGA
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mo-bullet79 TATCTATTTT TT...CACTA CATATTGCAG GAATTTCATC AATTTTAGGA
mjk-bullet49 TTTCTATTTT TT...CACTA CATATTGCAG GAATTTCATC AATTTTAGGA
mp-bullet69 TATCTATTTT TT...CACTA CATATTGCAG GAATTTCATC AATTTTAGGA
mj-mice51 TATCTATTTT TT...CACTT CATATTGCAG GAATTTCATC AATCTTAGGA
mjk-mice41 TATCTATTTT TT...CACTT CATATTGCAG GAATTTCATC AATCTTAGGA
mt-mice31 TATCTATTTT TT...CACTT CATATTGCAG GAATTTCATC AATCTTAGGA
mz-mice11 TATCTATTTT TT...CACTT CATATTGCAG GAATTTCATC AATCTTAGGA
mm-mice21 TATCTATTTT TT...CGCTT CATATTGCAG GAATTTCATC AATCTTAGGA
mt-bell32 TATCTATTTT TT...CTCTT CATATTGCAG GAATTTCATC AATTTTAGGA
mm-bell22 TATCTATTTT TT...CTCTT CATATTGCAG GAATTTCATC AATTTTAGGA
mjk-bell42 TATCTATTTT TT...CTCTT CATATTGCAG GAATTTCATC AATTTTAGGA
mt-club34 TATCTATTTT TT...CTCTT CATATTGCAG GAATTTCATC AATTTTAGGA
mo-club74 TATCTATTTT TT...CTCTT CATATTGCAG GAATTTCATC AATTTTAGGA
mp-club64 TATCTATTTT TT...CTCTT CATATTGCAG GAATTTCATC AATTTTAGGA
mk-bird83 TATCTATTTT TT...CCCTT CATATTGCAG GAATTTCATC AATTTTAGGA
mz-bell12 TATCTATTTT TT...CTCTT CATATTGCAG GAATTTCATC AATTTTAGGA
mj-club54 TATCTATTTT TT...CTCTT CATATTGCAG GAATTTCATC AATTTTAGGA
mjk-club44 TATCTATTTT TT...CTCTT CATATTGCAG GAATTTCATC AATTTTAGGA
mp-spindle68 TCTCTATTTT TT...CCCTT CATATTGCTG GAATTTCCTC TATCTTAGGA
mt-spindle38 TCTCTATTTT TT...CCCTT CATATTGCTG GAATTTCCTC TATCTTAGGA
mm-spindle28 TTTCAATTAT TT...CCCTC CATATAGCTG GAATTTCCTC TATTTTAGGA
mz-spindle18 TTTCAATTAT TT...CCCTC CATATAGCTG GAATTTCCTC TATTTTAGGA
mj-spindle58 TTTCTATTTT TT...CCCTC CATATTGCTG GAATTTCCTC TATTTTAGGA
mz-bud17 TTTCATTTTT TT...CACTT CATATAGCTG GAATTTCTTC TATTTTAGGA

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   mt-bud37 TCTCAATTTT TT...CTTTA CATATTGCTG GTATTTCCTC AATTTTAGGG

            201                                              250
Drosophila_b tgagctcatc atatattcac agtaggaata gatgtagaca cacgagctta
Drosophila_a tgagcccatc atatattcac ggtaggaata gatgtagaca ctcgagctta
Asphondylia_ gctattaatt ttattactac aattattaac ataaaaaata aatttattaa
Asphondylia_ gctattaatt ttattactac tatcattaat ataaaaaata aatttattaa
Asphondylia_ gcaattaatt ttattactac aattattaat ataaaaaata aatttattaa
mj-blister56 GCAATTAATT TTATTTCAAC TATATTTAAT ATAAAAATTA AATTTTTAAA
mo-blister76 GCGATTAATT TTATTTCAAC TATATTAAAT ATAAAAATTA AATTTTTAAA
mt-blister36 GCAATTAATT TTATTTCAAC TATATTTAAT ATAAAAATTA AATTTTTAAA
  mjk-bulb45 GCAATTAATT TTATTTCAAC AATTATAAAT ATAAAAAATA AAAATTTAAA
   mj-bulb55 GCAATTAATT TTATTTCAAC TATTATAAAT ATAAAAAATA AAAATTTAAA
   mm-bulb25 GCAATTAATT TTATCTCAAC AATTATAAAT ATAAAAAATA AAAATTTAAA
 mt-bullet39 TCTATAAATT TTATTTCTAC AATTATAAAT ATAAAAAATA TAAATTTAAA
 mj-bullet59 TCTATCAATT TTATTTCTAC AATTATAAAT ATAAAAAATA TAAATTTAAA
 mz-bullet19 GCTATCAATT TTATTTCTAC AATTATAAAT ATAAAAAATA ATAATTTAAA
 mm-bullet29 GCTATCAATT TTATTTCTAC AATTATAAAT ATAAAAAATA ATAATTTAAA
 mo-bullet79 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA ATAATTTAAA
mjk-bullet49 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA ATAATTTAAA
 mp-bullet69 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA ATAATTTAAA
   mj-mice51 GCTATTAATT TTATTTCTAC GATTATAAAT ATAAAAAACA AAAATTTAAA
  mjk-mice41 GCTATTAATT TTATTTCTAC GATTATAAAT ATAAAAAACA AAAATTTAAA
   mt-mice31 GCTATTAATT TTATTTCTAC GATTATAAAT ATAAAAAACA AAAATTTAAA
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    mz-mice11 GCTATTAATT TTATTTCTAC GATTATAAAT ATAAAAAACA AAAATTTAAA
    mm-mice21 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
    mt-bell32 GCAATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
    mm-bell22 GCAATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
   mjk-bell42 GCAATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
    mt-club34 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
    mo-club74 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
    mp-club64 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
    mk-bird83 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
    mz-bell12 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
    mj-club54 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
   mjk-club44 GCTATTAATT TTATTTCTAC AATTATAAAT ATAAAAAATA AAAATTTAAA
 mp-spindle68 GCAATTAATT TTATTTCAAC AATAATTAAT ATAAAAATTA AATTTCTAAA
 mt-spindle38 GCAATTAATT TTATTTCAAC AATAATTAAT ATAAAAATTA AATTTCTAAA
 mm-spindle28 GCAATTAATT TTATCTCAAC AATAATTAAT ATAAAAATTA AATATTTAAA
 mz-spindle18 GCAATTAATT TTATCTCAAC AATAATTAAT ATAAAAATTA AATATTTAAA
 mj-spindle58 GCAATTAATT TTATCTCAAC AATAATTAAT ATAAAAATTA AATATTTAAA
     mz-bud17 GCTATTAATT TTATTTCTAC TATTTTAAAT ATAAAAATTA AATTTATTAA
     mt-bud37 GCAATTAACT TTATTTCAAC AATACTAAAT ATAAAAATTA AATTTATTAA

              251                                                300
Drosophila_b ttttacttca gctactataa ttattgctgt acctacagga attaaaattt
Drosophila_a ctttacttct gctactataa ttattgctgt acctacagga attaaaattt
Asphondylia_ aattaatgaa ttatcacttt ttatctgatc aattttaatt actaccattc
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Asphondylia_	attaaatgaa	ctttcccttt	ttatttgatc	aattttaatt	actactgttc
Asphondylia_	atttaatgaa	atatcactat	ttatttgatc	aattctaatt	acaactattc
mj-blister56	ATTCGATCAA	ATTTCTTTAT	TTACATGATC	AGTACTAATT	ACAGCATTTT
mo-blister76	ATTTGATCAA	ATTTCTTTAT	TTATTTGATC	TATTATAATC	ACTACTATCC
mt-blister36	ATTTGACCAA	ATTTCTTTAT	TTACATGATC	AGTATTAATT	ACTGCATTTC
mjk-bulb45	ATTTAATAAA	TTATCTTTAT	TTATTTGATC	AATTTTAATT	ACAACTATTT
mj-bulb55	ATTTAATAAA	TTATCTTTAT	TTATTTGATC	AATTTTAATT	ACAACTATTT
mm-bulb25	ATTTAATAAA	TTATCTTTAT	TTATTTGATC	AATTTTAATT	ACAACTATTT
mt-bullet39	ATTTTATGAA	CTTTCTTTAT	TCATTTGATC	AATTCTCATT	ACATCAATTT
mj-bullet59	ATTTTATGAA	CTTTCTTTAT	TTATTTGATC	AATTCTTATT	ACATCAATTT
mz-bullet19	TTTTAATAAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATT	ACAACAATTT
mm-bullet29	TTTTAATAAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATT	ACAACAATTT
mo-bullet79	ATTTAATAAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATT	ACAACAATTT
mjk-bullet49	ATTTAATAAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATT	ACAACAATTT
mp-bullet69	ATTTAATAAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATT	ACAACAATTT
mj-mice51	ATTTAATGAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATC	ACAACAATTT
mjk-mice41	ATTTAATGAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATC	ACAACAATTT
mt-mice31	ATTTAATGAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATC	ACAACAATTT
mz-mice11	ATTTAATGAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATC	ACAACAATTT
mm-mice21	ATTTAATGAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATT	ACAACAATTT
mt-bell32	ATTTAATGAA	CTTTCTTTAT	TCATTTGATC	AATTTTTATT	ACAACAATCT
mm-bell22	ATTTAATGAA	CTTTCTTTAT	TCATTTGATC	AATTTTTATT	ACAACAATCT
mjk-bell42	ATTTAATGAA	CTTTCTTTAT	TCATTTGATC	AATTTTTATT	ACAACAATCT
mt-club34	ATTTAATGAA	CTTTCTTTAT	TTATTTGATC	AATTTTTATT	ACAACAATTT

```
   mo-club74 ATTTAATGAA CTTTCTTTAT TTATTTGATC AATTTTTATT ACAACAATTT
   mp-club64 ATTTAATGAA CTTTCTTTAT TTATTTGATC AATTTTTATT ACTACAATTT
   mk-bird83 ATTTAATGAA CTTTCTTTAT TTATTTGATC AATTTTTATT ACAACAATTT
   mz-bell12 ATTTAATGAA CTTTCTTTAT TCATTTGATC AATTTTTATT ACTACAATTT
   mj-club54 ATTTAATGAA CTTTCTTTAT TTATTTGATC AATTTTTATT ACTACAATTT
  mjk-club44 ATTTAATGAA CTTTCTTTAT TTATTTGATC AATTTTTATT ACTACAATTT
mp-spindle68 GATAGATCAA ATATCATTAT TTATTTGATC TATTTTAATT ACAACGATCT
mt-spindle38 GATAGATCAA ATATCATTAT TTATTTGATC TATTTTAATT ACAACAATCT
mm-spindle28 AATAGATCAA ATATCACTTT TTATTTGATC TATTTATATT ACAACAATTC
mz-spindle18 AATAGATCAA ATATCACTTT TTATTTGATC TATTTATATT ACAACAATTC
mj-spindle58 AATAGATCAA ATATCATTTT TTATTTGATC TATTTATATT ACAACAATTC
    mz-bud17 AGCTAATCAA ATTTCATTAT TTATTTGATC AATTATTATT ACAGCTATTT
    mt-bud37 ATTTGATCAA ATTTCTTTAT TCACATGATC AGTATTAATT ACTGCCATTC

             301                                               350
Drosophila_b ttagatgatt agctacttta catggggctc aactttcata ttcacctgct
Drosophila_a ttagttgatt agctactctt catggagctc aactaacata ttctccagct
Asphondylia_ ttttactttt atctttacca gttcttgcag gagcaatcac tatactaatt
Asphondylia_ ttttactttt atcacttcct gtacttgcag gagcaattac tatattatta
Asphondylia_ ttttattatt atcattacct gtattagctg gagcaattac tatattatta
mj-blister56 TATTATTATT ATCATTACCA GTTTTAGCTG GAGCTATTAC AATATTATTA
mo-blister76 TTTTACTCCT TTCTTTACCT ATTTTAGCAG GAGCTATTAC TATACTTTTA
mt-blister36 TTTTATTATT ATCTTTACCA GTATTAGCAG GAGCAATTAC AATATTATTA
   mjk-bulb45 TATTACTTTT ATCATTACCT GTTTTGGCCG GAGCTATTAC AATATTATTA
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mj-bulb55	TATTACTTTT	ATCATTACCT	GTTTTGGCCG	GAGCTATTAC	AATATTATTA
mm-bulb25	TATTACTTTT	ATCATTACCT	GTTTTGGCCG	GAGCTATTAC	AATATTATTA
mt-bullet39	TATTATTACT	ATCATTACCA	GTCTTAGCTG	GAGCAATTAC	AATATTATTA
mj-bullet59	TATTATTACT	ATCATTACCA	GTATTAGCTG	GAGCAATCAC	AATATTATTA
mz-bullet19	TATTATTACT	ATCCTTACCA	GTTTTAGCTG	GAGCAATCAC	AATATTGCTA
mm-bullet29	TATTATTACT	ATCCTTACCA	GTTTTAGCTG	GAGCAATCAC	AATATTACTA
mo-bullet79	TATTATTATT	ATCTTTACCA	GTTTTAGCTG	GAGCAATTAC	AATATTATTA
mjk-bullet49	TATTATTATT	ATCTTTACCA	GTTTTAGCTG	GAGCAATTAC	AATATTATTA
mp-bullet69	TATTATTATT	ATCTTTACCA	GTTTTAGCTG	GAGCAATTAC	AATATTATTA
mj-mice51	TATTATTATT	ATCTTTACCA	GTATTAGCTG	GAGCAATTAC	AATATTATTA
mjk-mice41	TATTATTATT	ATCTTTACCA	GTATTAGCTG	GAGCAATTAC	AATATTATTA
mt-mice31	TATTATTATT	ATCTTTACCA	GTATTAGCTG	GAGCAATTAC	AATATTATTA
mz-mice11	TATTATTATT	ATCTTTACCA	GTATTAGCTG	GAGCAATTAC	AATATTATTA
mm-mice21	TATTATTATT	ATCTTTACCA	GTTTTAGCTG	GAGCAATTAC	AATATTATTA
mt-bell32	TATTATTATT	ATCATTACCA	GTTTTAGCAG	GAGCAATTAC	AATATTATTA
mm-bell22	TATTATTATT	ATCATTACCA	GTTTTAGCAG	GAGCAATTAC	AATATTATTA
mjk-bell42	TATTATTATT	ATCATTACCA	GTTTTAGCAG	GAGCAATTAC	AATATTATTA
mt-club34	TATTATTATT	ATCATTACCA	GTTTTAGCTG	GAGCAATTAC	AATATTATTA
mo-club74	TATTATTATT	ATCATTACCA	GTTTTAGCTG	GAGCAATTAC	AATATTATTA
mp-club64	TATTATTATT	ATCATTACCA	GTTTTAGCTG	GAGCAATTAC	AATATTATTA
mk-bird83	TATTATTACT	ATCATTACCA	GTTTTAGCTG	GAGCAATTAC	AATATTATTA
mz-bell12	TATTGTTATT	ATCATTACCA	GTTTTAGCTG	GAGCAATTAC	AATATTATTA
mj-club54	TATTATTATT	ATCATTACCA	GTTTTAGCTG	GAGCAATTAC	AATACTATTA
mjk-club44	TATTATTATT	ATCATTACCA	GTTTTAGCTG	GAGCAATTAC	AATACTATTA

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mp-spindle68 TATTAATTAT TTCTTTACCT GTTTTAGCAG GAGCAATTAC TATATTATTA
mt-spindle38 TATTAATTAT TTCTTTACCT GTTTTAGCAG GAGCAATTAC TATATTATTA
mm-spindle28 TTTTATTACT TGCATTACCT GTTTTAGCAG GAGCAATTAC TATACTACTA
mz-spindle18 TTTTATTACT TGCATTACCT GTTTTAGCAG GAGCAATTAC TATACTACTA
mj-spindle58 TTTTATTACT TGCTTTACCT GTTTTAGCAG GAGCAATTAC TATACTACTA
     mz-bud17 TACTTCTTCT TTCATTACCT GTTTTAGCTG GAGCAATTAC TATATTATTA
     mt-bud37 TTTTATTATT ATCACTACCA GTTCTAGCCG GAGCAATTAC TATACTTCTT

             351                                              400
Drosophila_b attttatgag cattaggatt tgtattttta tttactgttg gaggattaac
Drosophila_a attttatgag cactaggatt cgtattttta tttactgttg gtggtttaac
Asphondylia_ actgaccgaa acttaaatac ttcatttttt gatcctatag gaggggtga
Asphondylia_ actgatcgaa atattaatac tacatttttt gacccaatag gaggaggaga
Asphondylia_ actgatcgaa atattaatac atcttttttt gatcctatag gaggggtga
mj-blister56 ATAGATCGAA ACTTAAATAC ATCATTCTTT GATCCTATAG GAGGAGGAGA
mo-blister76 ACTGATCGAA ATTTAAATAC ATCATTTTTT GACCCCATAG GAGGAGGAGA
mt-blister36 ATAGACCGTA ATTTAAATAC ATCATTTTTT GATCCAATAG GAGGAGGAGA
  mjk-bulb45 ACAGATCGAA ATATAAATAC ATCTTTTTTT GACCCACTCG GAGGAGGAGA
   mj-bulb55 ACAGATCGAA ATATAAATAC ATCATTTTTT GACCCACTCG GAGGAGGAGA
   mm-bulb25 ACAGATCGAA ATATAAATAC ATCATTTTTT GACCCACTCG GAGGAGGAGA
 mt-bullet39 ACTGATCGAA ATTTAAATAC TTCTTTTTTT GATCCTATTG GAGGAGGAGA
 mj-bullet59 ACTGATCGAA ATTTAAATAC TTCTTTTTTT GATCCTATTG GAGGAGGTGA
 mz-bullet19 ACTGATCGAA ATTTAAATAC ATCTTTTTTC GATCCATTAG GAGGTGGAGA
 mm-bullet29 ACTGATCGAA ATTTAAATAC ATCTTTTTTC GATCCATTAG GAGGTGGAGA
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mo-bullet79	ACTGATCGAA	ATTTAAATAC	ATCTTTCTTT	GACCCATTAG	GAGGTGGAGA
mjk-bullet49	ACTGATCGAA	ATTTAAATAC	ATCTTTCTTT	GACCCATTAG	GAGGTGGAGA
mp-bullet69	ACTGATCGAA	ATTTAAATAC	ATCTTTCTTT	GACCCATTAG	GAGGTGGAGA
mj-mice51	ACTGATCGAA	ATTTAAATAC	TTCATTTTTT	GACCCACTAG	GAGGAGGAGA
mjk-mice41	ACTGATCGAA	ATTTAAATAC	TTCATTTTTT	GACCCACTAG	GAGGAGGAGA
mt-mice31	ACTGATCGAA	ATTTAAATAC	TTCATTTTTT	GACCCACTAG	GAGGAGGAGA
mz-mice11	ACTGATCGAA	ATTTAAATAC	TTCATTTTTT	GACCCACTAG	GAGGAGGAGA
mm-mice21	ACTGATCGAA	ATTTAAATAC	TTCATTTTTT	GATCCTCTAG	GAGGGGGAGA
mt-bell32	ACAGATCGAA	ATTTAAATAC	TTCTTTTTTT	GATCCATTAG	GAGGAGGAGA
mm-bell22	ACAGATCGAA	ATTTAAATAC	TTCTTTTTTT	GATCCATTAG	GAGGAGGAGA
mjk-bell42	ACAGATCGAA	ATTTAAATAC	TTCTTTTTTT	GATCCATTAG	GAGGAGGAGA
mt-club34	ACAGATCGAA	ATTTAAACAC	TTCTTTTTTT	GATCCATTAG	GAGGAGGAGA
mo-club74	ACAGATCGAA	ATTTAAACAC	TTCTTTTTTT	GATCCATTAG	GAGGAGGAGA
mp-club64	ACAGATCGAA	ATTTAAACAC	TTCTTTTTTT	GATCCATTAG	GAGGAGGAGA
mk-bird83	ACAGATCGAA	ATTTAAATAC	TTCTTTTTTT	GATCCATTAG	GAGGAGGAGA
mz-bell12	ACAGATCGAA	ATTTAAATAC	TTCTTTTTTT	GATCCATTAG	GAGGAGGAGA
mj-club54	ACAGATCGAA	ATTTAAACAC	TTCTTTTTTT	GATCCATTAG	GAGGAGGAGA
mjk-club44	ACAGATCGAA	ATTTAAACAC	TTCTTTTTTT	GATCCATTAG	GAGGAGGAGA
mp-spindle68	ACAGATCGAA	ATCTAAACAC	ATCATTTTTC	GATCCTATAG	GAGGAGGAGA
mt-spindle38	ACAGATCGAA	ATCTAAACAC	ATCATTTTTC	GATCCTATAG	GAGGAGGAGA
mm-spindle28	ACAGATCGAA	ATCTAAATAC	ATCATTTTTT	GACCCAATAG	GAGGAGGAGA
mz-spindle18	ACAGATCGAA	ATCTAAATAC	ATCATTTTTT	GACCCAATAG	GAGGAGGAGA
mj-spindle58	ACAGATCGAA	ATCTAAATAC	ATCATTTTTT	GACCCAATAG	GAGGAGGAGA
mz-bud17	ACTGATCGAA	ATTTAAATAC	ATCATTTTTT	GATCCTATAG	GAGGAGGTGA

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 mt-bud37 ACTGATCGAA ATTTAAATAC ATCTTTTTTT GATCCAATAG GGGGAGGAGA

          401                                              450
Drosophila_b tggagtagtt ttagctaact cctctgttga tattattctt catgacactt
Drosophila_a aggagtagtc ttagctaact catctgttga tattattctt catgatacat
Asphondylia_ cccaattctt tatcaacatt tattttgatt ttttggtcat cct~~~~~~~
Asphondylia_ tccaattctt tatcaacatt tattttgatt ttttggtcat cct~~~~~~~
Asphondylia_ tcctattctt tatcaacatt tattttgatt ttgcggtcac cca~~~~~~~
mj-blister56 TCCAATTTTA TATCAACATT TATTTTGATT TTTTGGACAT CCAGAATTTT
mo-blister76 TCCATTTTTA TATCAACACT TATTTTGATT TTTTGGCCAC CCTGAATTTT
mt-blister36 TCCAATTTTA TATCAACATT TATTTTGATT TTTTGGTCAC CCAGAAGTTT
 mjk-bulb45 TCCAATTTTA TATCAACATT TATTTTGATT TTTTGGACAT CCAGAATTTT
  mj-bulb55 TCCAATTTTA TATCAACATT TATTTTGATT TTTTGGACAT CCAGAAGTTT
  mm-bulb25 CCCAATTTTA TACCAACATT TATTTTGATT TTTTGGACAC CCAGAAGTTT
mt-bullet39 TCCTATTCTA TATCAACATC TATTTTGATT TTTTGGACAT CCAGAAGTTT
mj-bullet59 TCCTATTCTT TATCAACATC TATTTTGATT TTTTGGACAT CCAGAAGTTT
mz-bullet19 TCCAATTTTA TATCAACATT TATTTTGATT TTTTGGCCAC CCAGAAGTTT
mm-bullet29 TCCAATTTTA TATCAACATT TATTTTGATT TTTTGGTCAC CCAGAAGTTT
mo-bullet79 TCCAATTTTA TATCAACATT TATTTTGATT TTTTGGTCAT CCAGAAGTTT
mjk-bullet49 TCCAATTTTA TATCAACATT TATTTTGATT TTTTGGTCAT CCAGAAGTTT
mp-bullet69 TCCAATTTTA TATCAACATT TATTTTGATT TTTTGGTCAT CCAGAAGTTT
  mj-mice51 TCCAATTCTT TATCAACATT TATTTTGATT CTTTGGACAT CCTGAAGTTT
 mjk-mice41 TCCAATTCTT TATCAACATT TATTTTGATT CTTTGGACAT CCTGAAGTTT
  mt-mice31 TCCAATTCTT TATCAACATT TATTTTGATT CTTTGGACAT CCTGAAGTTT
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mz-mice11      TCCAATTCTT TATCAACATT TATTTTGATT CTTTGGACAT CCTGAAGTTT
mm-mice21      TCCAATTCTT TATCAACATT TATTTTGATT CTTTGGACAT CCTGAAGTTT
mt-bell32      TCCAATTCTT TATCAACATT TATTTTGATT TTTTGGACAT CCAGAAGTTT
mm-bell22      TCCAATTCTT TATCAACATT TATTTTGATT TTTTGGACAT CCAGAAGTTT
mjk-bell42     TCCAATTCTT TATCAACATT TATTTTGATT TTTTGGACAT CCAGAAGTTT
mt-club34      TCCAATTCTT TATCAACATT TATTCTGATT TTTTGGGCAT CCAGAAGTTT
mo-club74      TCCAATTCTT TATCAACATT TATTCTGATT TTTTGGGCAT CCAGAAGTTT
mp-club64      TCCAATTCTT TATCAACATT TATTTTGATT TTTCGGACAT CCAGAAGTTT
mk-bird83      TCCAATTCTC TATCAACATT TATTCTGATT TTTTGGACAT CCAGAAGTTT
mz-bell12      TCCGATTCTT TATCAACATT TATTTTGATT TTTTGGACAT CCAGAAGTTT
mj-club54      TCCAATTCTT TATCAACATT TATTTTGATT TTTCGGACAT CCAGAAGTTT
mjk-club44     TCCAATTCTT TATCAACATT TATTTTGATT TTTCGGACAT CCAGAAGTTT
mp-spindle68   TCCTATTTTA TATCAACATT TATTTTGATT TTTTGGTCAT CCAGAAGTTT
mt-spindle38   TCCTATTTTA TATCAACATT TATTTTGATT TTTTGGTCAT CCAGAAGTTT
mm-spindle28   TCCTATTTTA TATCAACACT TATTTTGATT TTTTGGACAC CCAGAATTTT
mz-spindle18   TCCTATTTTA TATCAACACT TATTTTGATT TTTTGGACAC CCAGAAGTTT
mj-spindle58   TCCTATTTTA TATCAACACT TATTTTGATT TTTTGGACAC CCAGAAGTTT
mz-bud17       CCCAATTCTG TATCAACATT TATTTTGATT TTTTGGACAT CCAGAAGTTT
mt-bud37       TCCAGTATTA TACCAACATT TATTTTGATT CTTTGGTCAT CCTGAAGTTT

               451                                               500
Drosophila_b   attacgtagt tgcacatttt cattatgtgt tatctatggg agcagtattt
Drosophila_a   attatgtagt tgctcatttc cattatgtat tatctatagg agctgnatnt
Asphondylia_   ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
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 Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mj-blister56 ATATTTTAAT TGTTACCGGG A~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mo-blister76 ATATTTTAAT TGTTACCGGG ATGGGCCTGG TGGGTTATTT TGAACCAATT
 mt-blister36 ATATTTTAAT TTTACCGGGA TTT~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mjk-bulb45 ATATTTTAAT TTTACCGGGA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mj-bulb55 ATATTTTAAT TTTACCGGGA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mm-bulb25 ATATTTTATT TTTACCGGGA GGAAGTTTAT ~~~~~~~~~~ ~~~~~~~~~~
  mt-bullet39 ATATTTTATT TTTTCCGGGG GGG~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mj-bullet59 ATATTTTAAT TTTACCGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mz-bullet19 ATATTTTATT TTTTCCCCGG GGGG~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mm-bullet29 ATATTTTAAT TTTACCGGGG G~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mo-bullet79 ATATTTTAAT TTTTTCCGGG G~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
 mjk-bullet49 ATATTTTATT TTTTACCGGG GA~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mp-bullet69 ATATTTTAAT TTTTACCGGG GGGGG~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mj-mice51 ATATTTTAAT TTTTACCGGG GG~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mjk-mice41 ATATTTTAAT TTTTCCCGGG GGA~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mt-mice31 ATATTTTAAT TTTTCCGGGG AG~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mz-mice11 ATATTTATTT TTTTCCCCGG GGGA~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mm-mice21 ATATTTTATT TTTACCGGGG GG~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mt-bell32 ATATTTTAAT TTTCCCCGGG G~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mm-bell22 ATATTTTAAT TTTGCCCGGG GG~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mjk-bell42 ATATTTAATT TTTTCCCCGG GGGA~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mt-club34 ATATTTTAAT TTTTTCCGGG GA~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
```

```
   mo-club74 ATATTTTAAT TTTACCGGGG AN~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mp-club64 ATATTTTAAT TTTACCGGGA ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mk-bird83 ATATTTTAAT TTTTCCCCGG GGA~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mz-bell12 ATATTTTATT TTTTTCCCGG GGGG~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mj-club54 ATATTTTATT TT~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  mjk-club44 ATATTTTAAT TTTACCGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mp-spindle68 ATATTTTAAT TTTTACCGGG GA~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mt-spindle38 ATATTTTATT T~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mm-spindle28 ATATTTTAAT TTTACCGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mz-spindle18 ATATTTTAAT TTTACCGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
mj-spindle58 ATATTTTAAT TTTACCGGG~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mz-bud17 ATATTTTATT TTTTACGGGG ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
    mt-bud37 ATATTTAATT TTTTCCCGGG GA~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

             501                                   539
Drosophila_b gctat~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
Drosophila_a gctattatag cagg~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
Asphondylia_ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
mj-blister56 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
mo-blister76 TTTCGGTTCT TGGGGAGGGC TATATCAGGG GATACAACA
mt-blister36 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
   mjk-bulb45 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
```

mj-bulb55 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mm-bulb25 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mt-bullet39 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mj-bullet59 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mz-bullet19 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mm-bullet29 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mo-bullet79 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mjk-bullet49 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mp-bullet69 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mj-mice51 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mjk-mice41 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mt-mice31 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mz-mice11 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mm-mice21 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mt-bell32 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mm-bell22 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mjk-bell42 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mt-club34 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mo-club74 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mp-club64 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mk-bird83 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mz-bell12 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mj-club54 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~
mjk-club44 ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~~ ~~~~~~~~~~

```
mp-spindle68 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
mt-spindle38 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
mm-spindle28 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
mz-spindle18 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
mj-spindle58 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
    mz-bud17 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
    mt-bud37 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~
```

Appendix 7. 12S Gene UPGMA Genetic Distances

Genetic Distances

Calculated over: 65 to 328
Considering all base positions
Correction method: Kimura 2-parameter
Distances are: estimated number of substitutions per 100 bases
Symmatrix version 1
Number of matrices: 1

//
Matrix 1, dimension: 33

Key for column and row indices:

1 Drosophila_bifasciata_12S
2 Drosophila_ambigua_12S
3 mt-bud37
4 mz-bud17
5 mz-spindle18
6 mm-spindle28
7 mj-spindle58
8 mp-spindle68
9 Asphondylia_sphaera_12s
10 Asphondylia_itoi__12s

11 Asphondylia_gennadii_12s
12 mm-bulb25
13 mj-bulb55
14 mz-bulb15
15 mjk-mice41
16 mt-mice31
17 mj-mice51
18 mz-mice11
19 mm-mice21
20 mj-club54
21 mz-bell12
22 mt-bell32
23 mjk-bell42
24 mm-bell22
25 mo-club74
26 mt-club34
27 mt-bullet39
28 mj-bullet59
29 mz-bullet19
30 mm-bullet29
31 mjk-bullet49
32 mp-bullet69
33 mo-bullet79

Matrix 1: Part 1

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.00	6.79	147.23	144.00	138.07	138.07	142.64	124.11	138.47	168.81	114.98	95.98
2		0.00	173.42	165.94	164.24	164.24	177.74	139.83	157.84	999.99	121.29	100.01
3			0.00	-0.00	23.19	22.68	23.23	22.08	47.29	43.77	45.94	41.13
4				0.00	22.75	22.25	22.79	21.65	46.75	43.25	45.42	41.35
5					0.00	0.39	1.57	4.40	50.79	42.02	43.98	49.00
6						0.00	1.17	3.98	50.10	41.38	43.32	48.32
7							0.00	3.58	48.60	40.03	41.93	49.13
8								0.00	48.48	41.37	41.16	49.67
9									0.00	12.81	15.77	38.70
10										0.00	13.51	32.00
11											0.00	33.69
12												0.00
13												
14												
15												
16												
17												
18												
19												
20												

l 21 l

l 22 l

l 23 l

l 24 l

l 25 l

l 26 l

l 27 l

l 28 l

l 29 l

l 30 l

l 31 l

l 32 l

l 33 l

Matrix 1: Part 2

	13	14	15	16	17	18	19	20	21	22	23	24
1	89.53	91.74	90.10	95.20	114.10	113.87	113.89	117.69	109.89	112.29	113.54	113.56
2	93.22	92.07	90.87	96.07	115.55	115.29	115.29	117.95	115.30	112.32	113.56	113.61
3	39.10	46.11	37.32	36.07	36.01	37.58	34.25	33.55	35.64	32.57	33.14	32.74
4	39.31	46.36	37.52	36.27	36.20	37.79	34.43	33.72	35.83	32.74	33.32	32.91
5	47.18	59.00	47.87	46.59	48.10	48.67	45.92	40.85	43.23	42.55	43.18	42.79
6	46.54	58.28	47.17	45.91	47.41	47.98	45.26	40.85	43.23	42.55	43.18	42.79
7	47.36	59.26	47.06	45.79	47.28	47.83	45.13	41.60	44.01	43.32	43.96	43.57
8	47.91	58.63	44.79	43.55	46.59	48.52	42.93	43.68	46.17	45.45	46.09	45.71
9	37.90	46.99	40.44	33.70	33.03	37.09	34.37	35.64	39.73	38.01	39.44	38.32
10	31.20	43.89	37.35	32.19	31.53	35.53	30.36	32.09	34.15	32.59	33.93	32.86
11	33.80	45.30	39.50	35.49	33.99	36.65	32.86	35.44	38.83	35.79	37.20	36.09
12	4.04	16.09	28.46	26.12	27.86	29.08	22.94	26.59	29.05	26.34	26.89	26.51
13	0.00	11.95	26.38	24.10	25.24	26.58	21.02	26.57	28.53	26.28	26.82	26.49
14		0.00	23.19	26.65	36.59	37.52	34.14	38.87	39.95	38.05	38.64	38.40
15			0.00	0.81	10.83	11.82	9.90	21.25	21.94	21.02	21.59	21.69
16				0.00	5.00	6.33	7.19	18.08	19.24	17.29	17.80	17.91
17					0.00	4.62	8.54	16.48	17.60	16.69	17.20	17.30
18						0.00	10.88	19.16	20.34	19.42	19.95	20.06
19							0.00	14.30	15.89	14.98	16.01	15.56
20								0.00	2.82	2.02	3.26	2.44

21	0.00	4.09	5.37	4.53
22		0.00	1.20	0.40
23			0.00	1.62
24				0.00
25				
26				
27				
28				
29				
30				
31				
32				
33				

Matrix 1: Part 3

	25	26	27	28	29	30	31	32	33
1	117.71	109.16	101.01	101.96	91.74	92.52	94.47	95.20	93.43
2	117.96	108.99	106.36	105.18	88.74	89.49	99.92	100.90	98.85
3	35.14	36.71	40.88	39.66	34.93	34.93	40.66	40.76	39.42
4	35.33	36.91	41.10	39.87	34.44	34.44	40.16	40.25	38.92
5	42.18	44.90	49.89	49.57	42.48	42.72	44.88	44.10	43.99
6	42.18	44.90	49.20	48.89	41.83	42.07	44.22	43.45	43.34

7	42.94	45.71	48.36	48.03	41.83	42.07	45.00	44.22	44.10
8	45.03	46.26	47.57	45.60	40.21	40.44	44.89	44.10	43.98
9	40.47	41.66	37.85	34.49	32.42	32.42	33.49	33.55	32.33
10	30.40	34.04	33.12	31.73	32.18	32.18	29.06	28.44	29.06
11	37.41	39.99	33.41	31.15	28.33	28.33	28.30	27.69	28.99
12	26.80	27.57	27.20	26.45	22.64	22.19	25.36	25.36	24.26
13	26.75	27.58	23.00	22.79	22.37	21.91	22.34	22.36	21.30
14	39.16	37.34	34.62	34.49	35.88	35.40	33.15	33.15	32.57
15	23.10	21.53	24.56	26.15	27.06	26.58	25.33	25.33	25.89
16	19.27	17.77	20.70	22.16	22.46	21.99	22.51	22.55	22.51
17	17.65	19.37	20.63	22.05	22.34	21.87	22.42	22.44	22.42
18	19.34	21.13	22.94	24.44	23.11	22.64	24.25	24.28	24.25
19	15.91	16.53	17.34	17.60	18.37	17.91	18.94	18.97	18.94
20	3.26	3.68	22.88	22.68	19.05	18.60	21.16	21.20	21.16
21	5.37	4.94	24.69	24.51	21.81	21.35	22.92	22.96	22.92
22	4.09	4.52	23.73	23.54	20.34	19.88	20.95	20.99	20.95
23	4.94	5.80	25.42	24.70	21.46	21.00	22.06	22.10	22.06
24	4.53	4.96	24.42	24.24	21.00	20.54	21.60	21.65	21.09
25	0.00	3.69	25.88	24.47	21.35	20.90	21.40	21.45	21.40
26		0.00	26.64	25.89	22.59	22.13	22.67	22.72	22.67
27			0.00	2.88	13.38	13.44	14.60	14.61	14.60
28				0.00	12.83	12.88	12.66	12.67	13.62
29					0.00	-0.00	13.67	13.68	14.16
30						0.00	13.74	13.74	14.23

31	0.00	0.40	2.00
32		0.00	2.00
33			0.00

```
#NEXUS

begin trees;
tree Tree_1 = (('Drosophila_bifasciata_12S':3.39,'Drosophila_ambigua_12S':3.39)
:61.30,((('mt-bud37':-0.00,'mz-bud17':-0.00):11.29,((('mz-spindle18'
:0.19,'mm-spindle28':0.19):0.49,'mj-spindle58':0.69):1.31,
'mp-spindle68':1.99):9.30):12.93,((('Asphondylia_sphaera_12s'
:6.41,'Asphondylia_itoi__12s':6.41):1.25,'Asphondylia_gennadii_12s'
:7.66):12.56,((('mm-bulb25':2.02,'mj-bulb55':2.02):4.99,'mz-bulb15'
:7.01):7.88,((((('mjk-mice41':0.41,'mt-mice31':0.41):3.84,
('mj-mice51':2.31,'mz-mice11':2.31):1.94):0.32,'mm-mice21'
:4.56):4.61,((('mj-club54':1.37,(('mt-bell32':0.20,'mm-bell22'
:0.20):0.51,'mjk-bell42':0.71):0.67):0.71,'mz-bell12':2.08)
:0.31,('mo-club74':1.85,'mt-club34':1.85):0.55):6.78):1.78,
((('mt-bullet39':1.44,'mj-bullet59':1.44):5.17,('mz-bullet19'
:-0.00,'mm-bullet29':-0.00):6.61):0.39,(('mjk-bullet49':0.20,
'mp-bullet69':0.20):0.80,'mo-bullet79':1.00):6.00):3.95)
:3.93):5.33):4.00):40.48):0.00;
endblock;
```

```
Symbol comparison table: pileupdna.cmp  CompCheck: 6876

                   GapWeight: 5
             GapLengthWeight: 1

PileUp  MSF: 423  Type: N  March 17, 2008 00:02  Check: 8150 ..

 Name: Drosophila_bifasciata_12S  Len:   423  Check: 8398  Weight:  1.00
 Name: Drosophila_ambigua_12S  Len:   423  Check: 6871  Weight:  1.00
 Name: mt-bud37         Len:   423  Check: 4335  Weight:  1.00
 Name: mz-bud17         Len:   423  Check: 8697  Weight:  1.00
 Name: mz-spindle18     Len:   423  Check: 3515  Weight:  1.00
 Name: mm-spindle28     Len:   423  Check: 4247  Weight:  1.00
 Name: mj-spindle58     Len:   423  Check: 6862  Weight:  1.00
 Name: mp-spindle68     Len:   423  Check: 8524  Weight:  1.00
 Name: Asphondylia_sphaera_12s  Len:   423  Check: 7909  Weight:  1.00
 Name: Asphondylia_itoi__12s  Len:   423  Check:  932  Weight:  1.00
 Name: Asphondylia_gennadii_12s  Len:   423  Check: 7522  Weight:  1.00
 Name: mm-bulb25        Len:   423  Check: 4012  Weight:  1.00
 Name: mj-bulb55        Len:   423  Check: 1299  Weight:  1.00
 Name: mz-bulb15        Len:   423  Check: 9622  Weight:  1.00
 Name: mjk-mice41       Len:   423  Check: 2153  Weight:  1.00
 Name: mt-mice31        Len:   423  Check: 1619  Weight:  1.00
 Name: mj-mice51        Len:   423  Check: 4845  Weight:  1.00
```

```
Name: mz-mice11         Len:   423  Check: 5135  Weight:  1.00
Name: mm-mice21         Len:   423  Check: 3616  Weight:  1.00
Name: mj-club54         Len:   423  Check: 2388  Weight:  1.00
Name: mz-bell12         Len:   423  Check: 3472  Weight:  1.00
Name: mt-bell32         Len:   423  Check: 2621  Weight:  1.00
Name: mjk-bell42        Len:   423  Check: 7095  Weight:  1.00
Name: mm-bell22         Len:   423  Check: 4975  Weight:  1.00
Name: mo-club74         Len:   423  Check: 5971  Weight:  1.00
Name: mt-club34         Len:   423  Check: 7708  Weight:  1.00
Name: mt-bullet39       Len:   423  Check: 2217  Weight:  1.00
Name: mj-bullet59       Len:   423  Check: 4263  Weight:  1.00
Name: mz-bullet19       Len:   423  Check: 3254  Weight:  1.00
Name: mm-bullet29       Len:   423  Check:  866  Weight:  1.00
Name: mjk-bullet49      Len:   423  Check: 9049  Weight:  1.00
Name: mp-bullet69       Len:   423  Check: 4332  Weight:  1.00
Name: mo-bullet79       Len:   423  Check: 9826  Weight:  1.00

//
                1                                                   50
Drosophila_b ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~aaaat ttattattaa tttgatttat
Drosophila_a ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~aaaat ttattattaa tttgatttat
    mt-bud37 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ CTTAAATTAG
    mz-bud17 ~~~~~~~~~~ ~~~~AGAAAA TAACAAGACT GGGCATATGT ACATATTTTT
mz-spindle18 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGTGC GGGCATTTGT ACATATTATT
```

```
mm-spindle28 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGTGC GGGCATTTGT .CATATTATT
mj-spindle58 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~GGTGC GGGCATTTGT ACATATTATT
mp-spindle68 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~C GGGCATTTGT ACATATTATT
Asphondylia_ ~~~tttaaaa ttaattaaaa gcgacgggca atatgtatat attattttaa
Asphondylia_ ~~~tttaaaa ttaattaaaa gcgacgggca atatgtatat attattttaa
Asphondylia_ ~~~tttaaaa ttaattaaaa gcgacgggcg atatgtatgt attactttta
   mm-bulb25 ~~~~~~~~~~ ~~~~~~~~~~ ~CCGATTGGG AAATGAATAT ATTAAAAATA
   mj-bulb55 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
   mz-bulb15 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~NNGGGG GGGGCNCGNT GCCGGTNCAG
  mjk-mice41 ~~~~~~~~~~ ~~~~~~~~TG GCATATGAAT ATATTAATAA AATAAATTTA
   mt-mice31 ~~~~~~~~~~ ~~~~~~~~~G GCAGATGAAT ATATTAATAA AATAAATTTA
   mj-mice51 ~~~~~~~~~~ ~~~~~~~~GG GCATGGGCAT ATATTAGTAA AATAAATTTA
   mz-mice11 ~~~~~~~~~~ ~~~~~TGTTG GCATTGGAAT ATATTAATAA AATAAATTTA
   mm-mice21 ~~~~~~~~~~ ~~~~~~~~~~ ~~~TATGAAT ATATTAATAA AATAAATTTA
   mj-club54 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~GAAT ATATTTATAA TTTAAATTTA
   mz-bell12 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~AATT ATATTTATAA TTTAAATTTA
   mt-bell32 ~~~~~~~~~~ ~~~~~GGTAG GCATATGGAT ATATTTATAA TTTAAATTTA
  mjk-bell42 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~AT ATATTTATAA TTTAAATTTA
   mm-bell22 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~TGAT ATATTTATAA TTTAAATTTA
   mo-club74 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~CTTTATAA TTTAAATTTA
   mt-club34 ~~~~~~~~~~ ~~~~~~~~~~ ~CATATGAAT ATATTTATAA TTTAAATTTA
 mt-bullet39 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~GTAATTA AATTATCTAA
 mj-bullet59 ~~~~~~~ACC GGGAACTAGG GCAATATGAA TATATTTATA ATATATATTA
 mz-bullet19 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~CAAAT AATCATCTGA
```

```
  mm-bullet29 ~~~CATCCAA AAAATTCGGC TTTATTAAAA TATATTAATA AAATATATTA
 mjk-bullet49 ~~~~~~~GAC TAAATTTAAG GCATATGAAT ATATTAATAA ATATATATTA
  mp-bullet69 ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~AAAAAAAT TATCATATTA
  mo-bullet79 AGGAAAAAAT TCTAAATAGT CAATATGAAT ATATTAATAA ATATATATTA

              51                                               100
Drosophila_b  atgtaaattt ttgtatgaat ttatatttat tttaaaaata tttataattt
Drosophila_a  atgtaaattt ttgtgtgaaa ttatatttat tttaaaaata tttataatat
    mt-bud37  CATGTCCATT TAATTATAAA ATTTATTATA ATTTAATTTT AAATACCATT
    mz-bud17  TACATTCATT TAATTATAAA ATTTATTATA ATTTAATTTT AAAT.CCATT
 mz-spindle18 AAAAATTATT CAAATTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
 mm-spindle28 AAAAATTATT CAAATTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
 mj-spindle58 AAAAATTATT CAATTTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
 mp-spindle68 AAAAATTATT CAATTTATAA ATTTATATAA ATTTAATTTT AAATTCAATT
 Asphondylia_ aatattaaaa ttttaaacct ataaaatttt aattttaaat ccattttcat
 Asphondylia_ aatattaaat tattaaattt ataataattt aattttaaat ccattttat
 Asphondylia_ aatattaaat atttaaatat ataaatattt aattttaaat ccaatttcat
   mm-bulb25  ATATTAAATT TTAACTATAT ATTAAAATTT AATATTACCC TCCAACGTTT
   mj-bulb55  ~~~~~~~~~~ ~~~~~TATAT ATTAAAATTT AATATTA..A ATCCAACTTT
   mz-bulb15  CTTANNGNTC NTNNGGATAT ATTAACAATT TA.ATATTAA ATCCAACTTT
  mjk-mice41  AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
   mt-mice31  AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
   mj-mice51  AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
   mz-mice11  AACATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
```

```
   mm-mice21 AATATAAAAT ATATTAATAA TTTTAATATT AAATCCAATT TTATAATTAT
   mj-club54 AAATAAATAT TTATTTATAA .TTTAATATT AAATCCAATT TAAAAAACAT
   mz-bell12 AAATAAATAT TTATTTATAA .TTTAATATT AAATCCAATT TTAAAAATAT
   mt-bell32 AAAAAAATAT TTA.TTATAA .TTTAATATT AAATCCAATT TAAAAAATAT
  mjk-bell42 AAAAAAATAT TTA.TTATAA .TTTAATATT AAAGCCAATT TAAAAAATAT
   mm-bell22 AAAAAAATAT TTA.TTATAA .TTTAATATT AAAT.CAATT TAAAAAATAT
   mo-club74 AAATAAATAT TTA.TTATAA .TTTAAGATT AAATCCAATT TAAAAAATAT
   mt-club34 AAATAAATAT TTA.TTATAA .TTTAATATT AAATCCAATT TAAAAAATAT
 mt-bullet39 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATATAACTTA
 mj-bullet59 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATATAACTTA
 mz-bullet19 AAAAATAATA TATATTAAAA .TTTAATATT AAATCCAACT ACTATAAATT
 mm-bullet29 AAAAATAATA TATATTAAAA .TTTAATATT AAATCCAACT ACTATAAATT
mjk-bullet49 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATTTTAATTT
 mp-bullet69 AAAAATAATA TATATTATTT .TTTAATATT AAATCCAATT ATTTTAATTT
 mo-bullet79 AAAAATAATA TATATTATTT .TTTAATATT AAATTCAATT ATTTTAATTT

             101                                              150
Drosophila_b atttattcgc agtaattagt attattaatt aaagaaattt agaaatagca
Drosophila_a atttattagc agtaattaat attataaatt aaagaaattt agaaatagca
    mt-bud37 TTCAAAAATT TATTACAAAA ATTTATTTAA AATTTATATT AATGTATCTC
    mz-bud17 TTCAAAAATT TATTACAAAA ATTTATTTAA AATTTATATT AATGTATCTC
mz-spindle18 TCATAA.TTT TTTTACAAAA ATTAATTCAA AAACATTTTT ATTGTATTTC
mm-spindle28 TCATAA.TTT TTTTACAAAA ATTAATTCAA AAATATTTTT ATTGTATTTC
mj-spindle58 TCATAA.TTT TTTTACAAAA TTTAATTCAA AAATATTTTT ATTGTATTTC
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mp-spindle68 TCATAATTTT TTTTACAAAA TTTAATTCAA AAATATTTTT ATTGTATTTC
Asphondylia_ ttaaatatta aaatttaaaa ttcaatttta aaatttattt aatgtatttc
Asphondylia_ ttaaatatta aaatttaaaa tccaatt... aaatttattt aatgtatttc
Asphondylia_ ttaaatatta aaacttaaaa tcc.atatat aaataaattt aatgtatttc
   mm-bulb25 ATTAATATAT AACAATAAAA ATTCTGCAAA AATTAAAATA AATGTATTTC
   mj-bulb55 ATTAATATAT AACAATAAAA ATTCTGCAAA AATTAAAATA AATGTATTTC
   mz-bulb15 ATTAGTATAT AACAATAAAA ATTCTGCAGA ACTTAAAATA AATGTATTTC
  mjk-mice41 ATT....TAC AATAATAATG CTATAT.AAA A..TAATATT AATGTATTTC
   mt-mice31 ATT....TAC AATAATAATG CTATAT.AAA A..TAATGTT AATGTATTTC
   mj-mice51 ATT....TAC AATAATAATG CTATAT.AAA A..TAGTATT AATGTATTTC
   mz-mice11 ATT....TAC AATAATAATG CTGTAT.AAA A..TAATATT AATGTAGTTC
   mm-mice21 ATT....TAC AATAATAATG CTATATCCAA A..TAAGAAT AATGTATTTC
   mj-club54 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AATGTATTTC
   mz-bell12 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AAGGTATTTC
   mt-bell32 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AATGTATTTC
  mjk-bell42 TTTACAATAT AA.AATCCAT AGAAATTATA A..AATTATT AATGTATTTC
   mm-bell22 TTTACAATAT AA.AATCCAT AAAAATTATA A..AATTATT AATGTATTTC
   mo-club74 CTTACAATAT AAGAAGCCAT ACGAATTATA A..AATTATT AATGTATTTC
   mt-club34 CTTACAATAT AA.AATCCAT AAGACTTATA A..AATTATT AAGGTATTTC
 mt-bullet39 TATTACAATA AATAATAATA TAATACCATT A..TAAATCA AATGTATTTA
 mj-bullet59 TATTACAATA AATATAATAA TTA....ATT A..TAAATCA AATGTATTTC
 mz-bullet19 TATTACAATA AATACTAAAT .......AAA A..TAAATTT AATGTATTTC
 mm-bullet29 TATTACAATA AATACTAAAT .......AAA A..TAAATTT AATGTATTTC
mjk-bullet49 ATTACAATAA ATTATTAAAT ATA...AAAT A..TAAATTA AATGTATTTC
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  mp-bullet69 ATTACAATAA ATTATTAAAT ATA...AAAT A..TAAATTA AATGTATTTC
  mo-bullet79 ATTACAATAA ATTAATAAAT ATA...AAAT A..TAAATTA AATGTATTTC

              151                                                200
Drosophila_b atattaaaga gtattgacca aattggtgcc agcagtcgcg gttacactaa
Drosophila_a atattaaaga gtattgacca aattggtgcc agcagtcgcg gttacactaa
    mt-bud37 A.TTTAAAAT TTAAATATAA AATGAATTAT GATTTGACAT TTTATATT.A
    mz-bud17 A.TTTAAAAT TTAAATATAA AATGAATTAT GATTTGACAT TTTATATT.A
 mz-spindle18 A.TTTTATTT TTATATATTT AATGAATTTT GATTTGACTT TAAATTTAAA
 mm-spindle28 A.TTTTATTT TTATATATTT AATGAATTTT GATTTGACTT TAAATTTAAA
 mj-spindle58 A.TTTTATTT TTATATATTT AATGAATTTT GATTTGACTT CAAATTTAAA
 mp-spindle68 A.TTTTAATT TTATATATTT AATGAATTTT GATTTGACTT CTAATCTTAA
 Asphondylia_ atttt.aatc ttatactttt aatatattat gatttgaaat aaattaat..
 Asphondylia_ attttaaacc ttaaattttt aatatattat gatttgaatt aaattata..
 Asphondylia_ atttttaatc ttttatttta aatatattat gatttgaatt aaattaat..
    mm-bulb25 A.TTTAAAAT TTAATTATTA AATATATTAT GATTTGAATT TTGTTATAT.
    mj-bulb55 A.TTTAAAAT TTAATTATTA AATATATTAT GATTTGAATT TTAATATAT.
    mz-bulb15 A.TTTAAAAT TTAATTATTA AATATATTAT GATTTGAATT TTACTATATA
   mjk-mice41 A.TTTAAATC TTAAATATGA AATATATTAT GATTTGAAAT TTA..TTAT.
    mt-mice31 A.TTTAAATC TTAAATATGA AATATATTAT GATTTGAAAT TTA..TTAT.
    mj-mice51 A.TTTAAATC TTAAATATGA AATATATTAT GATTTGAAAT TTA..TTAT.
    mz-mice11 A.TTTAAATC TTAAATATGA AATATATTAT GATGTGAAGT TTA..TTAT.
    mm-mice21 A.TTTAAATC TTAAATATAA AATATATTAT GATTTGAAAT TTATTTTTT.
    mj-club54 A.TTTAAATT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
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    mz-bell12 A.TTTAAATT TTAAATATAA AATATATTAG GATTGGAAAT TATTTTAAT.
    mt-bell32 A.TTTAAAAT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
   mjk-bell42 A.TTTAAAAT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
    mm-bell22 A.TTTAAAAT TTAAATATAA AAGATATTAT GATTTGAAAT TATTTTAAT.
    mo-club74 A.TTTAAACT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
    mt-club34 A.TTTAAACT TTAAATATAA AATATATTAT GATTTGAAAT TATTTTAAT.
  mt-bullet39 A.TTTAAATC TTAAATATAA AATATATTAT AATTTGAAAT TTA.T.TAA.
  mj-bullet59 A.TTTAAATC TTAAATATAA AATATATTAT AATTTGAAAT TTATT.TTA.
  mz-bullet19 A.TCTAAATC TTAAATATAA AATATATTAT GATTTGAATT TTTTTAATA.
  mm-bullet29 A.TCTAAATC TTAAATATAA AATATATTAT GATTTGAATT TTTTTAAT..
 mjk-bullet49 A.TTTAAACC TTAAATATAA AATATATTAT GATTTGAAAT TAATTACTA.
  mp-bullet69 A.TTTAAACC TTAAATATAA AATATATTAT GATTTGAAAT TAATTACTA.
  mo-bullet79 A.TTTAAACC TTAAATATAT AATATATTAT GATTTGAAAT TAATTACTA.

              201                                            250
 Drosophila_b taatacaaat aaattttttt agtattagtt aaatttataa attaaaagaa
 Drosophila_a taatacaaat aaattttttt agtagtagtt aaatttattt attaaaataa
      mt-bud37 TTCAAATATT TAAATAATAA ATTTTTTAAA AATTATTTTA TGACAACAAT
      mz-bud17 TTCAAATATT TAAATAATAA ATTTTTTAAA AATTATTTTA TGACAACAAT
  mz-spindle18 ATTAAATTTT TCAATAATAA A..TCCAAAA AATTATTTTA AGACAACAAT
  mm-spindle28 ATTAAATTTT TCAATAATAA A..TCCAAAA AATTATTTTA AGACAACAAT
  mj-spindle58 ATTAAATTTT TCAATAATAA A..TCCAAAA AATTATTTTA AGACAACAAT
  mp-spindle68 ATTAAATTTT TTAATAATAA A..TTTAAAA AATTATTTTA AGACAACAAT
  Asphondylia_ .aaaaataaa atttattaat tttttattac aaaattaatt aaacaacaat
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Asphondylia_ .ataaaataa ttattaataa attttattat aaaattaatt aaacaacaat
Asphondylia_ ....aatata atatttatta atattttact aaaatcaatt aaacaacaat
   mm-bulb25 ..AAAAAATA AAATTAATTT TTTATAAAAT AAAATTAATT AAACAACAAT
   mj-bulb55 .AAAAAAATA AAATTAATTT TTTATAAAAT AAAATTAATT AAACAACAAT
   mz-bulb15 AAAANAAANA AANTAATTTT TTNAAAAANA AAANTNATTN AACCNCCCNT
  mjk-mice41 .AAAAAAANA NTANTAANAN TTTTTT...N AAANTNATTN AACCACCATN
   mt-mice31 .AAAAAAAAA ATTATAAAAA TTTTTT...T AAAATTANTT AACCACCANT
   mj-mice51 .AAAAAAAAT ATTATTAATA TTTTTT...T AAAATTAATT AAACAACAAT
   mz-mice11 ..AAAAAAAT ACTATTAATA TTTTTT...T AAAATTAATT AAACAACAAT
   mm-mice21 .AAAAAAATA TTATTAATAT TTTTTT...T AAAATTAATT AAACAACAAT
   mj-club54 .AAAAATTTT TAATTAATAT TTT.TTTATT AAAATTAATT AAACAACAAT
   mz-bel112 .AAAAATTTT TAATTAATA. TTT.TTTATT AAAATTAATA AAACAACAAT
   mt-bel132 .AAAAATATT TAATTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
  mjk-bel142 .AAAAATATT TAAGTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
   mm-bel122 .AAAAATATT TAATTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
   mo-club74 .AAAAAATTT TAATTAATA. ..T.TTTATT AAAATTAATT AAACAACAAT
   mt-club34 .AAAAATTTT TAATTAATA. ..T.TTTATT AAAATTAATT AACCAACAAT
 mt-bullet39 .AAAAAAATA AAATTAATTT TTTATT.AAT AAAATCAATT AAACAACAAT
 mj-bullet59 .AAAAAAATA AAATTAATTT TTTATT.AAT AAAATCAATT AAACAACAAT
 mz-bullet19 .AAAAAAATA AAATTAATTT TTTATTAAAT AAAATTAATT AAACAACAAT
 mm-bullet29 .AAAAAAATA AAATTAATTT TTTATTAAAT AAAATTAATT AAACAACAAT
mjk-bullet49 .ATAAAAATA AAATTAATTT TCTATTTAAT AAAATCAATT AAACAACAAT
 mp-bullet69 .ATAAAAATA AAATTAATTT TCTATTTAAT AAAATCAATT AAACAACAAT
 mo-bullet79 .ACAAAAATA AAATTAATTT TCTATTTAAT AAAATCAATT AAACAACAAT
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              251                                                300
Drosophila_b aattgaattt attaagtgaa attttatatt caaaatattt tta.taaaaa
Drosophila_a aatt.aattt attaagtgaa attttaaatt taaaatattt ttatttaaga
    mt-bud37 ATACAATTTA ATT.AATTTA AATATATTTT ATTGTGTATT ATC.AATTAA
    mz-bud17 ATACAATTTA ATT.AATTTA AATATATTTT ATTGTGTATT ATC.AATTAA
 mz-spindle18 ATACAATTTA ATT.AATATA AATAAATTAT AATGTGGATT ATC.AATTAA
 mm-spindle28 ATACAATTTA ATT.AATATA AATAAATTAT AATGTGGATT ATC.AATTAA
 mj-spindle58 ATACAATTTA ATT.AATATA AATAAATTAT AATGTGGATT ATC.AATTAA
 mp-spindle68 ATACAATTTA ATT.AATATA AATAAATTAT AAGGTGGATT ATC.AATTAA
 Asphondylia_ atataattta a.ttaatata agtttattta aatgtgtatt atcaaattaa
 Asphondylia_ atataatttt a.ttaaatta agtatatttt aatgtgtatt atc.aattaa
 Asphondylia_ atataatttt atttaataaa agtatattat aatgtgtatt atc.aattaa
    mm-bulb25 ATATAATTTT ATTAAA.TTA AATATATTAA ATTGGGGATT ATC.AATTAA
    mj-bulb55 ATATAATTTT ATTAAA.TTA AATATATTAA ATTGGGGATT ATC.AATTAA
    mz-bulb15 NNNAANTTTT ATNANA.TTA ANANTATAAA NTGGGGTATN ACC.ANTNAA
  mjk-mice41 NNTNANCCTN ANAAANTTNA AAAANNTAAA NTGGGGANTA ACC.ANTNAN
   mt-mice31 NTNTAANCTN AANAAATTNA AANANNTNAN ATGGGGAATN ANC.AATNAA
   mj-mice51 ATATAATCTT AATAAATTTA AATATATTGT ATTGTGTATT ATC.AATTAA
   mz-mice11 ATATAATCTC AATAAATTTA AATATATTAT ATTGTGTGTG ATC.AATTAA
   mm-mice21 ATATAATTTT AATAAATTTA AATATATTAT ATTGGGGATT ATC.AATTAA
   mj-club54 ATATAATTTT AATAAA.TTA AATATATTAT ATTGTGTATT ATC.AATTAA
   mz-bell12 ATATAATTTT AATAAA.TTA AATATATTAT GTTGTGTATT ATC.AATTAA
   mt-bell32 ATATAATTTT AATAAA.TCA AACATATTAT ATTGTGTATT ATC.AATTAA
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   mjk-bell42 ATATAATTTT AATAAA.TCA AACATATTAT ATTGTGTATT ATC.AATTAA
    mm-bell22 ATATAATTTT AATAAA.TCA AACATATTAT ATTGTGTATT ATC.AATTAA
    mo-club74 ATATAATTTT AATAAA.TTA AATATATTAT ATTGTGTATT ATC.AATTAA
    mt-club34 ATATAATTTT AATAAA.TTA AATATATTAT ATGGGGTATT ATC.AATTAA
  mt-bullet39 ATATAATTTT A.TAAA.ATA AATATATTAT AACGTGTATT ATC.TATTAA
  mj-bullet59 ATATAATTTT A.TAAA.ATA AATATATTAT AACGTGTATT ATC.TATTAA
  mz-bullet19 ATATAATTTA A.TAAA.ATA AATATATTAT AATGTGTATT ATC.AATTAA
  mm-bullet29 ATATAATTTA A.TAAA.ATA AATATATTAT AATGTGTATT ATC.AATTAA
 mjk-bullet49 ATATAATTTA ATTAAA.ATA AGTATATTAT AATGTGTATT ATC.AATTAA
  mp-bullet69 ATATAATTTA ATTAAA.ATA AGTATATTAT AATGTGTATT ATC.AATTAA
  mo-bullet79 ATATAATTTA ATTAAA.ATA AGTATATTAT AATGTGTATT ATC.AATTAA

              301                                              350
Drosophila_b ataattgaag ctaaaaaatt ttttaaaa~~ ~~~~~~~~~~ ~~~~~~~~~~
Drosophila_a ttgattgaag ctaaaaaatt tttgaaaa~~ ~~~~~~~~~~ ~~~~~~~~~~
     mt-bud37 TTAACAAGAT CCTCTAATTT TAAAAAATAC TGCCAATTTA TTTAATTTTT
     mz-bud17 TTAACAAGAT CCTCTAATTT TAAAAAATAC TGCCAATTTA TTTAATTTTT
 mz-spindle18 TAAACAAAAT CCTTTAATTT TTAAAAATAC TGCCATTTTA TTTAATTTTA
 mm-spindle28 TAAACAAAAT CCTTTAATTT TTAAAAATAC TGCCATTTTA TTTAATTTTA
 mj-spindle58 TAAACAAAAT CCTCTAATTT TTAAAAATAC TGCCACTTTA TTTAATTTTA
 mp-spindle68 TAAACAAAAT CCTTTAATTT TTAAAAATAC TGCCATTTTA TTTAATTTTT
 Asphondylia_ ttaacaaaat cctctaattt taaataatac taccaaatta tttaatttt.
 Asphondylia_ taaacaaaat cctctaattt taaataatac taccaaatta tttaatttt.
 Asphondylia_ taaacaaaat cctctaattt taaataatac caccaaatta tttaattttc
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mm-bulb25	TTAACAAAAT	CCCCTAATTT	TTAAAAA.TA	CTACCAAATT	AATTAATTTT
mj-bulb55	TTAACAAAAT	CCTCAAATTT	TTAAAAA.TA	CTACCAAATT	AATTAATTTT
mz-bulb15	TNACCAAAAC	CCCCNANTTT	TAAAAANGNN	CCNCNAAATN	AATTANTTTC
mjk-mice41	TNACCAAANC	CCCCNANTTT	TNAAAANNCC	NCCCAANTNA	NTNANTTTTN
mt-mice31	TTANCAAANN	CCNCTAATTT	TNAAAAANNC	NNCCAAATNA	ATNANTTTTN
mj-mice51	TTAACAAAAT	CCTCTAATTT	TTAAGAATAC	TACCAAATTA	ATTAAGTTTT
mz-mice11	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	ATTAATTTTT
mm-mice21	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	ATTAATTTTT
mj-club54	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mz-bell12	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mt-bell32	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mjk-bell42	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mm-bell22	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAGATTA	TTTAATTTTA
mo-club74	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mt-club34	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mt-bullet39	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mj-bullet59	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTA
mz-bullet19	TTGACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTT
mm-bullet29	TTGACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTT
mjk-bullet49	TCAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	CTTAATTTTT
mp-bullet69	TAAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	ATTAATTTTT
mo-bullet79	TTAACAAAAT	CCTCTAATTT	TTAAAAATAC	TACCAAATTA	TTTAATTTTT

351 400

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  Drosophila_b ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
  Drosophila_a ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~
      mt-bud37 TTTAAATTAA AAATTAATTT TTTTAA...A AAAAAAAAGG GGATCTAATC
      mz-bud17 TTTAAATTAA AAATTAATTT TTTTAA...A AAAAAAAAGG GTATCTAAAC
  mz-spindle18 TTATAAT.TT TTACTAATTA TTTTTA...A TCTTAAAATT AAAATAATAG
  mm-spindle28 TTATAAT.TT TTACTAATTA TTTTTA...A TCTTAAAATT AAAATAATAG
  mj-spindle58 ATCAATC.TA CTACTAATTA TTTATT...A TCATTAAAAT AATAGGGTAG
  mp-spindle68 ATATATTATT TTACTAATTA TTTATT...A AAATTAAAAT AATAGGGTAT
  Asphondylia_ ..taaaaata aaaatttact aattaaaaa. aaaattaatc cttataata~
  Asphondylia_ ..ttttaatt taataataat tattaaaaat ttaataaatt tatataata~
  Asphondylia_ aaaaaaaatt taatattact tattaaaata ttattaaact aatataata~
     mm-bulb25 CTCTTTTTAT AGA.ATAATA GGCCAA..TA AAT.TATATT ATTATAATAG
     mj-bulb55 CTCTTTTAAT AGA.ATAATA ATTAAT..AA ACTCTATATT ATTATAATAG
     mz-bulb15 CNCTTTTAAN AAA.ATANNA ATTAA....N AANTCNTATT ATNANAGNGG
    mjk-mice41 AAAANANTTT TNA.ANANTN ANAN.....C CCTNAAAAAA TTNNNANAGG
     mt-mice31 AANAANATTT TNA.AAAATT AANA.....N CCNTAAAAAA NTTATANNNG
     mj-mice51 AATAATATTT TTA.ATAATT AATA.....T CCATAAAAAA ATTATAATAG
     mz-mice11 AATAATATTT TTA.ATAATT AATA.....T CCATAAAAAA ATTATAATAG
     mm-mice21 AATATCATAT TTA.ATAATT AGTA.....T TCATAAAAAA ATTATAATAG
     mj-club54 AAATAAAAAA TTA.ATAATT AAATAT...A TAATAAAAAA ATTATAATAG
     mz-bell12 AAATAAAAAA TTA.ATAATT AAATATATAA TAATAAAAAA ATTAGAATAG
     mt-bell32 AAATAAAAAA ATT.AATAAT TAAATT...T ATATATAAAA ATTATAATAG
    mjk-bell42 AAATAAAAAA ATT.AATAAT TAAATT...T ATATATAAAA ATTATAATAG
     mm-bell22 AAATAAAAAA ATT.AATAAT TAAATT...T ATATATAAAA ATTATAATAG
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mo-club74	AAATAAAAAT	TTA.ATAATT	AAATAT...A	TAATAAAAAA	ATTATAATAG
mt-club34	AAATAAAAAT	TTA.ATAATT	AAATAT...A	TAATAAAAAA	ATTATAATAG
mt-bullet39	ATTTTTTAAA	TTA.ATAATT	AATA.....C	AAATAAAAAA	ATTATAATAG
mj-bullet59	ATTTTTTAAA	TTA.ATAATT	AATA......	GAATAAAAAA	ATTATAATAG
mz-bullet19	A.TTTTAATA	TTA.ATAATT	AATA.....A	AA..AAAAAT	ATTATAATAG
mm-bullet29	A.TTTTAATA	TTA.ATAATT	AATA.....A	AA..AAAAAT	ATTATAATAG
mjk-bullet49	A.CTTTAAAA	TTA.ATAATT	AATA.....T	AATTAAAAAA	ATTATAATAG
mp-bullet69	A.TTTTAAAA	TTA.ATAATT	AATA.....T	AATTAAAAAA	ATTATAATAG
mo-bullet79	A.TTTTAAAA	TTA.ATAATT	AATA.....T	AATTAAAAAA	ATTATAATAG

	401		423
Drosophila_b	~~~~~~~~~~	~~~~~~~~~~	~~~
Drosophila_a	~~~~~~~~~~	~~~~~~~~~~	~~~
mt-bud37	CTAGTTTAGT	CA~~~~~~~~	~~~
mz-bud17	CTAATTTA~~	~~~~~~~~~~	~~~
mz-spindle18	GGTATCTAAT	CCCTAGTTTA	~~~
mm-spindle28	GGTATCTAAT	CCTAGTTTA~	~~~
mj-spindle58	GGAATCCTAG	CCTA~~~~~~	~~~
mp-spindle68	CTAATCCTAG	TTTA~~~~~~	~~~
Asphondylia_	~~~~~~~~~~	~~~~~~~~~~	~~~
Asphondylia_	~~~~~~~~~~	~~~~~~~~~~	~~~
Asphondylia_	~~~~~~~~~~	~~~~~~~~~~	~~~
mm-bulb25	GGCATCTAAT	CCTAGTTTA~	~~~
mj-bulb55	GGGATCTAAT	CCTAGTTTAA	~~~

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  mz-bulb15 GGNACCNANC CCNNNNTAAN ~~~
 mjk-mice41 GGANCCNANC CCAGGTTAA~ ~~~
  mt-mice31 GGNANCTAAC CCNNGTTAA~ ~~~
  mj-mice51 GGGATCTAAT CCTAGTTTA~ ~~~
  mz-mice11 GGGGGCTAAT CCTAGTTTAT ATA
  mm-mice21 GGGATCTAAT CCTAGTTTA~ ~~~
  mj-club54 GGTATCTAAT CCTAGTTTA~ ~~~
  mz-bell12 GGTATCTAAT CCAAGTTTA~ ~~~
  mt-bell32 GGTATCTAAT CCTAGTTTA~ ~~~
 mjk-bell42 GGTATCTAAT CCTAGTTTA~ ~~~
  mm-bell22 GGTATCTAAT CCTAGTTTA~ ~~~
  mo-club74 GGTATCTAAT CCTAGTTTA~ ~~~
  mt-club34 GGTATCTAAC CCTAGTTTA~ ~~~
 mt-bullet39 GGTATCTAAT CCTAGTTTAA ~~~
 mj-bullet59 GGTATCTAAT CCTAGTTTAA ~~~
 mz-bullet19 GGTATCTAAT CCTAGTTTAA ~~~
 mm-bullet29 GGTATCTAAT CCTAGTTTAA ~~~
mjk-bullet49 GGTATCTAAT CCTAGTTTAA ~~~
 mp-bullet69 GGTATCTAAT CCAAGTTTAA ~~~
 mo-bullet79 GGTATCTAAT CCTAGTTTAA ~~~
```

Appendix 8. Protein identification by MASCOT

Mascot Search Results

Protein View

Match to: gi|3851512 Score: 356

ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit [Trichocladus crinitus]

Found in search of C:\Documents and Settings\ABCD\×ÀÃæ\for ê ø™±ó\for —îÎ¬œW\050322\050322 Eing-1.pkl

Nominal mass (M_r): 52004; Calculated pI value: 5.96

NCBI BLAST search of gi|3851512 against nr

Unformatted sequence string for pasting into other applications

Taxonomy: Trichocladus crinitus

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: 12%

Matched peptides shown in **Bold Red**

```
  1 ETKASVGFKA GVKDYKLTYY TPQYETKDTD ILAAFRVTPQ PGVPPEEAGA
 51 AVAAESSTGT WTTVWTDGLT SLDRYKGXCY HIEPVAGEET QFIAYVAYPL
101 DLFEEGSVTN MFTSIVGNVF GFKALXALRL EDLRIPPAYS KTFQGPPHGX
151 QVERDKLNKY GRPLLGCTIK PKLGLSAKNY GRAVYECLRG GLDFTKDDEN
201 VNSQPFMRWR DRFLFCAEXX YKAQAETGEI KGHYLNATAG TCEEMMKRAV
251 FARELGVPIV MHDYLTGGFT ANTSLAHYCR DNGLLLHIHR AMHAVIDRQK
```

301 NHGMHFRVLA KALRMSGGDH IHAGTVVGKL EGEREITLGF VDLLRDDVIE
351 KDRSRGIYFT QDWVSLPGVL PVASGGIHVW HMPALTEIFG DDSVLQFGGG
401 TLGHPWGNAP GAVANRVALE ACVQARXEGR DLAREGXEII REASKWSPEL
451 AAACEVWKEI KFEFEAMDTL

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
10 - 16	390.8681	779.7216	779.4177	0.3039	1	K.AGVKDYK.L (Ions score 17)
130 - 141	468.1093	1401.3061	1400.7663	0.5397	1	R.LEDLRIPPAYSK.T (Ions score 22)
135 - 141	388.3773	774.7400	774.4276	0.3125	0	R.IPPAYSK.T (Ions score 23)
135 - 141	388.3781	774.7416	774.4276	0.3141	0	R.IPPAYSK.T (Ions score 38)
135 - 141	388.3805	774.7464	774.4276	0.3189	0	R.IPPAYSK.T (Ions score 34)
135 - 141	388.3873	774.7600	774.4276	0.3325	0	R.IPPAYSK.T (Ions score 12)
142 - 154	733.6090	1465.2034	1465.7062	-0.5027	0	K.TFQGPPHGNQVER.D (Ions score 74)
142 - 154	489.4322	1465.2748	1465.7062	-0.4314	0	K.TFQGPPHGNQVER.D (Ions score 66)
197 - 208	726.5422	1451.0698	1450.6147	0.4552	0	K.DDENVNSQPFMR.W (Ions score 38)
431 - 441	643.5540	1285.0934	1285.6626	-0.5691	1	R.DLAREGDEIIR.E (Ions score 45)
435 - 441	406.8755	811.7364	812.4392	-0.7027	0	R.EGPEIIR.E (Ions score 34)
435 - 441	415.8811	829.7476	829.4293	0.3183	0	R.EGNEIIR.E (Ions score 46)
435 - 441	415.8840	829.7534	830.4134	-0.6599	0	R.EGDEIIR.E (Ions score 33)
435 - 445	623.5291	1245.0436	1244.6360	0.4076	1	R.EGNEIIREASK.W (Ions score 41)

Mascot Search Results

Protein View

Match to: gi|57338572 Score: 208
ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Persea americana]
Found in search of C:\Documents and Settings\ABCD\×ÀÃæ\for ê ø™±ó\for —îÎ¬œW\050322\050322 Eing-2.pkl
Nominal mass (M_r): 26807; Calculated pI value: 8.54
NCBI BLAST search of gi|57338572 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Persea americana

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: 25%

Matched peptides shown in **Bold Red**

```
  1 KGTKASVGFK AGVKDYKLTY YTPDYETKST DILAAFRVTP QPGVPPEEAG
 51 AAVAAESSTG TWTTVWTDGL TSLDRYKGRC YHIEPVAGEE SQFIAYVAYP
101 LDLFEEGSVT NMFTSIVGNV FGFKALRALR LEDLRIPPAY SKTFQGPPHG
151 IQVERDKLNK YGRPLLGCTI KPKLGLSAKN YGRAVYECLR GGLDFTKDDE
201 NVNSQPFMRW RDRFLFCAEA IYKSQAETGE IKGHYLNATA GK
```

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
18 - 28	697.5219	1393.0292	1392.6449	0.3844	0	K.LTYYTPDYETK.S (Ions score 39)

29 - 37	497.4302	992.8458	992.5291	0.3168	0	K.STDILAAFR.V (Ions score 38)
136 - 142	388.3681	774.7216	774.4276	0.2941	0	R.IPPAYSK.T (Ions score 32)
143 - 155	489.4069	1465.1989	1464.7473	0.4516	0	K.TFQGPPHGIQVER.D (Ions score 47)
198 - 209	726.5072	1450.9998	1450.6147	0.3852	0	K.DDENVNSQPFMR.W (Ions score 25)
224 - 232	481.9134	961.8122	961.4716	0.3406	0	K.SQAETGEIK.G (Ions score 29)

Mascot Search Results

Protein View

Match to: gi|37992949 Score: 164

ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit [Persea americana]

Found in search of C:\Documents and Settings\ABCD\×ÀÃæ\for ê ø™±ó\for —îÍ¬œW\050322\050322 Eing-3.pkl

Nominal mass (M_r): 50306; Calculated pI value: 6.04

NCBI BLAST search of gi|37992949 against nr

Unformatted sequence string for pasting into other applications

Taxonomy: Persea americana

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: 12%

Matched peptides shown in **Bold Red**

```
  1 LTYYTPDYET KSTDILAAFR VTPQPGVPPE EAGAAVAAES STGTWTTVWT
 51 DGLTSLDRYK GRCYHIEPVA GEESQFIAYV AYPLDLFEEG SVTNMFTSIV
101 GNVFGFKALR ALRLEDLRIP PAYSKTFQGP PHGIQVERDK LNKYGRPLLG
151 CTIKPKLGLS AKNYGRAVYE CLRGGLDFTK DDENVNSQPF MRWRDRFLFC
201 AEAIYKSQAE TGEIKGHYLN ATAGTCEEMI KRAVFARELG VPIVMHDYLT
251 GGFTANTTLA HYCRDNGLLL HIHRAMHAVI DRQKNHGMHF RVLAKALRMS
301 GGDHIHAGTV VGKLEGERDI TLGFVDLLRD DFIEKDRSRG IYFTQDWVSM
351 PGVLPVASGG IHVWHMPALT EIFGDDSVLQ FGGGTLGHPW GNAPGAVANR
```

401 VALEACVQAR NEGRDLAREG NEIIREASKW SPELAAACEV WKEIKFEFAA
451 MDTL

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
1 - 11	697.4260	1392.8374	1392.6449	0.1926	0	-.LTYYTPDYETK.S (Ions score 27)
12 - 20	497.3566	992.6986	992.5291	0.1696	0	K.STDILAAFR.V (Ions score 30)
119 - 125	388.3166	774.6186	774.4276	0.1911	0	R.IPPAYSK.T (Ions score 46)
126 - 138	489.3410	1465.0012	1464.7473	0.2539	0	K.TFQGPPHGIQVER.D (Ions score 23)
207 - 215	481.8500	961.6854	961.4716	0.2138	0	K.SQAETGEIK.G (Ions score 19)
419 - 425	415.8206	829.6266	829.4293	0.1973	0	R.EGNEIIR.E (Ions score 22)

Mascot Search Results

Protein View

Match to: gi|18831 Score: 245

mitochondrial ATP synthase beta-subunit [Hevea brasiliensis]

Found in search of C:\Documents and Settings\ABCD\×ÀÃæ\for ê ø™±ó\for —îÎ¬ŒW\050322\050322 Eing-4.pkl

Nominal mass (M_r): 60221; Calculated pI value: 5.95

NCBI BLAST search of gi|18831 against nr

Unformatted sequence string for pasting into other applications

Taxonomy: Hevea brasiliensis

Links to retrieve other entries containing this sequence from NCBI Entrez:

gi|231586 from Hevea brasiliensis

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: 12%

Matched peptides shown in **Bold Red**

```
  1 MASRRLLSSL LRSSSRRSVS KSPISNINPK LSSSSPSSKS RASPYGYLLT
 51 RAAEYATSAA AAAPPQPPPA KPEGGKGGGK ITDEFTGKGA IGQVCQVIGA
101 VVDVRFDEGL PPILTSLEVL DHSIRLVLEV AQHMGEGMVR TIAMDGTEGL
151 VRGQRVLNTG SPITVPVGRA NPWTYHEVIG EPIDERGDIK TSHFLPIHRE
201 APAFVDQATE QQILVTGIKV VDLLAPYQRG GKIGLFGGAG VGKTVLIMEL
251 INNVAKAHGG FSVFAGVGER TREGNDLYRE MIESGVIKLG DKQADSKCAL
```

301 VYGQMNEPPG ARARVGLTGL TVAEHFRDAE GQDVLLFIDN IFRFTQANSE
351 VSALLGRIPS AVGYQPTLAT DLGGLQERIT TTKKGSITSV QAIYVPADDL
401 TDPAPATTFA HLDATTVLSR QISELGIYPA VDPLDSTSRM LSPHILGEEH
451 YNTARGVQKV LQNYKNLQDI IAILGMDELS EDDKLTVARA RKIQRFLSQP
501 FHVAEVFTGA PGKYVELKES ITSFQGVLDG KYDDLPEQSF YMVGGIDEVI
551 AKADKIAKES AS

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
141 - 152	631.8638	1261.7130	1261.6336	0.0795	0	R.TIAMDGTEGLVR.G (Ions score 59)
156 - 169	705.4551	1408.8956	1408.8038	0.0919	0	R.VLNTGSPITVPVGR.A (Ions score 62)
220 - 229	587.3928	1172.7710	1172.6553	0.1157	0	K.VVDLLAPYQR.G (Ions score 35)
344 - 357	746.9310	1491.8474	1491.7681	0.0794	0	R.FTQANSEVSALLGR.I (Ions score 64)
358 - 378	729.4227	2185.2463	2185.1378	0.1084	0	R.IPSAVGYQPTLATDLGGLQER.I (Ions score 26)

Mascot Search Results

Protein View

Match to: gi|3033513 Score: 84
rubisco activase [Phaseolus vulgaris]
Found in search of C:\Documents and Settings\ABCD\×ÀÃæ\for ê ø™±ó\for —îÍ¬œW\050322\050322 Eing-6.pkl
Nominal mass (M_r): 48171; Calculated pI value: 8.19
NCBI BLAST search of gi|3033513 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Phaseolus vulgaris
Links to retrieve other entries containing this sequence from NCBI Entrez:
gi|10720248 from Phaseolus vulgaris

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: 4%

Matched peptides shown in **Bold Red**

```
  1 MAASLSTVGA VNRTLLNLNG SGGGASGPSS AFFGTSLKKV ISSRVPNSKL
 51 TSGSFKIVAA DKEIEETQQT EGDRWRGLAY DVSDDQQDIT RGKGLVDSLF
101 QAPMDAGTHY AVISSHKYLS AGLRQYNFDN IKDGFYIAPA FLDKLVVHIA
151 KNFMTLPNIK VPLILGVWGG KGQGKSFQCE LVFAKMGINP IMMSAGELES
201 GNAGEPAKLI RQRYREASDL IKKGKMCVLF INDLDAGAGR LGGTTQYTVN
251 NQMVNATLMN IADNPTNVQL PGMYNKEDNA RVPIIVTGND FSTLYAPLIR
```

301 DGRMEKFYWA PTREDRIGVC KGIFRTDGVP EKDIVELVDK HPGQSIDFFG
351 ALRARVYDDE VRKWISGVGV DSVGKKLVNS KEGPPTFDQP KMTLDKLLLY
401 ASMLVQEQEN VKRVQLADQY LNEAALGNAN EDAIKSGSFF K

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
77 - 91	849.3645	1696.7144	1694.7747	1.9397	0	R.GLAYDVSDDQQDITR.G (Ions score 46)
356 - 362	448.2432	894.4718	894.4083	0.0636	0	R.VYDDEVR.K (Ions score 39)

Mascot Search Results

Protein View

Match to: gi|19157 Score: 78

33kDa precursor protein of oxygen-evolving complex [Lycopersicon esculentum]

Found in search of C:\Documents and Settings\ABCD\×ÀÃæ\for ê ø™±ó\for —îÎ¬œW\050322\050322 Eing-8.pkl

Nominal mass (M_r): 34926; Calculated pI value: 5.91

NCBI BLAST search of gi|19157 against nr

Unformatted sequence string for pasting into other applications

Taxonomy: Lycopersicon esculentum

Links to retrieve other entries containing this sequence from NCBI Entrez:

gi|12644171 from Lycopersicon esculentum

gi|738944 from Lycopersicon esculentum

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: 5%

Matched peptides shown in **Bold Red**

```
  1 MAASLQAAAT LMQPTKVGVR NNLQLRSAQS VSKAFGVEQG SGRLTCSLQT
 51 EIKELAQKCT DAAKIAGFAL ATSALVVSGA NAEGVPKRLT YDEIQSKTYM
101 EVKGTGTANQ CPTIEGGVGS FAFKPGKYTA KKFCLEPTSF TVKAEGVSKN
151 SAPDFQKTKL MTRLTYTLDE IEGPFEVSPD GTVKFEEKDG IDYAAVTVQL
201 PGGERVPFLF TIKQLVASGK PESFSGEFLV PSYRGSSFLD PKGRGGSTGY
```

251 DNAVALPAGG RGDEEELQKE NVKNTASLTG KITLSVTQSK PETGEVIGVF
301 ESIQPSDTDL GAKVPKDVKI QGIWYAQLE

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
88 - 97	626.8218	1251.6290	1251.6459	-0.0168	1	K.RLTYDEIQSK.T (Ions score 22)
89 - 97	548.7874	1095.5602	1095.5448	0.0155	0	R.LTYDEIQSK.T (Ions score 41)
235 - 242	425.7391	849.4636	849.4232	0.0404	0	R.GSSFLDPK.G (Ions score 15)

Mascot Search Results

Protein View

Match to: gi|3914592 Score: 135
Ribulose bisphosphate carboxylase small chain, chloroplast precursor (RuBisCO small subunit)
Found in search of C:\Documents and Settings\ABCD\×ÀÃæ\for ê ø™±ó\for —îÎ¬œW\050322\050322 Eing-11.pkl
Nominal mass (M_r): 20434; Calculated pI value: 8.88
NCBI BLAST search of gi|3914592 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Betula pendula
Links to retrieve other entries containing this sequence from NCBI Entrez:
gi|1536889 from Betula pendula

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: 12%

Matched peptides shown in **Bold Red**

```
  1 MACSMISSAT VAAVSRASPA QSSMVAPFTC LKSTSAFPVT QKTNNDITSI
 51 ASNGGRVQCM QVWPPLGLKK FETLSYLPPL SSEQLAKEVD YLLRKNLIPC
101 LEFELEHGFV YREHNRSPGY YDGRYWTMWK LPMFGCNDSS QVLKELEECK
151 KAYPSAFIRI IGFDNKRQVQ IISFIAYKPP GV
```

Start - End Observed Mr(expt) Mr(calc) Delta Miss Sequence

88 - 94	454.2487	906.4828	906.4810	0.0018	0	K.EVDYLLR.K	(Ions score 36)
117 - 124	457.7090	913.4034	913.3930	0.0105	0	R.SPGYYDGR.Y	(Ions score 59)
117 - 124	457.7101	913.4056	913.3930	0.0127	0	R.SPGYYDGR.Y	(Ions score 59)
160 - 167	481.7757	961.5368	961.5345	0.0024	1	R.IIGFDNKR.Q	(Ions score 43)

Mascot Search Results

Protein View

Match to: gi|9796478 Score: 45
serine threonine kinase homolog COK-4 [Phaseolus vulgaris]
Found in search of C:\Documents and Settings\ABCD\×ÀÃæ\for ê ø™±ó\for —ìÍ¬œW\050322\050322 Eing-23.pkl
Nominal mass (M_r): 42255; Calculated pI value: 6.42
NCBI BLAST search of gi|9796478 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Phaseolus vulgaris

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: 2%

Matched peptides shown in **Bold Red**

```
  1 MFLNCVGMCC SKPTTNTTSS QRQFPTLIEE LCHQFSLTDL RKATNNFDQK
 51 RVIGSGLFSE VYKGCLQHDG ASDYTVAIKR FDYQGWAAFN KEIELLCQLR
101 HPRCVSLIGF CNHENEKILV YEYMSNGSLD KHLQEGQLSW KKRLEICIGV
151 ARGLHFLHTG AKRSIFHCIL GPGTVLLDDQ MEPKLAGFDA SEQGSRFMSK
201 QKQINVIVFW VIFVLLYELT HCHDFLWIKL SLLFVIGCRG YTATDYLMDG
251 IITAKWDVFS FGFLLLEVVC RRMFYLITLT KKECLENPVE ERIDPIIKGK
301 IAPDCWQVFV DMMVSCLKYE PDERPTIGEV EVQLEHALSM QEQSDITNSN
351 SEYTLLSKTI ISLGVKKCK
```

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
144 - 152	487.4143	972.8140	972.5426	0.2715	0	R.LEICIGVAR.G (Ions score 45)

Mascot Search Results

Protein View

Match to: gi|24473812 Score: 46

small ribosomal subunit protein 4 [Bryum argenteum]

Found in search of C:\Documents and Settings\ABCD\×ÀÃæ\for ê ø™±ó\for —îÌ¬œW\050322\050322 Eing-26.pkl

Nominal mass (M_r): 23235; Calculated pI value: 10.51

NCBI BLAST search of gi|24473812 against nr

Unformatted sequence string for pasting into other applications

Taxonomy: Bryum argenteum

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Sequence Coverage: 6%

Matched peptides shown in **Bold Red**

```
  1 YRGPRVRIIR RLGALPGLTN KAPQLKTNSI NQSIFNKKIS QYRIRLEEKQ
 51 KLRFHYGITE RQLLNYVRIA RKAKGSTGEV LLQLLEMRLD NVIFRLGMAP
101 TIPGARQLVN HRHILVNDRI VNIPSYRCKP EDSITIKDRQ KSQAIISKNL
151 NLYQKYKTPN HLTYNFLKKK GLVNQILDRE SIGLKINELL VVEYYSRQA
```

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
27 - 37	633.4969	1264.9792	1264.6411	0.3381	0	K.TNSINQSIFNK.K (Ions score 28)
27 - 38	465.3969	1393.1689	1392.7361	0.4328	1	K.TNSINQSIFNKK.I (Ions score 18)

Mascot Search Results

Protein View

Match to: gi|7573598 Score: 54
protein kinase 2 [Populus nigra]
Found in search of C:\Documents and Settings\ABCD\×ÀÃæ\for ê ø™±ó\for —îÍ¬œW\050322\050322 Eing-27.pkl
Nominal mass (M_r): 45305; Calculated pI value: 7.11
NCBI BLAST search of gi|7573598 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Populus nigra

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: 1%

Matched peptides shown in Bold Red

```
  1 MGFLCFSGKS SKRSENSSID ENNSNIKRKD QTQLTSGSMK VKPYVNDSRE
 51 EGASKDDQLS LDVKSLNLKD EISKDIRNNG NPAQTFTFEE LVAATDNFRS
101 DCFLGEGGFG KVYKGYLEKI NQVVAIKQLD QNGLQGIREF VVEVLTLSLA
151 DNPNLVKLIG FCAEGDQRLL VYEYMPLGSL ENHLHDIPPN RQPLDWNARM
201 KIAAGAAKGL EYLHNEMAPP VIYRDLKCSN ILLGEGYHPK LSDFGLAKVG
251 PSGDHTHVST RVMGTYGYCA PDYAMTGQLT FKSDVYSFGV VLLELITGRK
301 AIDQTKERSE QNLVAWARPM FKDRRNFSGM VDPFLQGQYP IKGLYQALAI
351 AAMCVQEQPN MRPAVSDVVL ALNYLASHKY DPQIHPFKDP RRRPSHPGLD
```

401 KDNGRT

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
70 - 77	487.5033	972.9920	974.5032	-1.5112	1	K.DEISKDIR.N (Ions score 54)

Appendix 9. Protein sequence analysis and identification

Spot No.	Protein ID	Protein Name	Identified sequence	Species	Mowse Score	Source	Interesting reason	Figure No.
1	gi|3851512	RuBisCO large subunit	AGVKDYK/IPPAYSK/TFQGPPHG NQVER/DLAREGXEIIREASK	Trichocladus crinitus	356	MT leaf	All both galls<<leaves	Figure 5-10
2	gi|57338572	RuBisCO large subunit	LTYYTPDYETKSTDILAAFR/IPPA YSKTFQGPPHGIQVER/DDENVN SQPFMR/SQAETGE IK	Persea americana	208	MT leaf	All both galls<<leaves	Figure 5-10
3	gi|37992949	RuBisCO large subunit	LTYYTPDYETKSTDILAAFR /IPPAYSKTFQGP PHGIQVERDK /SQAE TGEIK/EG NEIIR	Persea americana	164	MT leaf	All both galls<<leaves	Figure 5-10
4	gi|18831	mitochondrial ATP synthase beta-subunit	TIAMDGTEGLVR/VLNTGSPITVP VGR/VVDLLAPYQR/FTQANSEV SALLGR/IPSAVGYQPTLATDLG GLQER	Hevea brasiliensis	245	MT leaf	MT mouse gall>> MZZ/MZM mouse galls	Figure18/19
5	gi|18403457	lipid binding	TTAVSLPR	Arabidopsis thaliana	30	MT leaf	All cup galls<<leaves	Figure 5/7/9
6	gi|16471	rubisco activase	GLAYDTSDDQQDITR/VYDDEVR	Arabidopsis thaliana	152	MT leaf	All cup galls<<leaves	Figure 5/7/9
7	gi|18403457	lipid binding	TTAVSLPR	Arabidopsis thaliana	32	MT leaf	All both galls<<leaves	Figure 5-10
8	gi|19157	33kDa precursor protein of oxygen-evolving complex	RLTYDEIQSK/GSSFLDPK	Lycopersicon esculentum	78	MT leaf	All both galls<<leaves	Figure 5-10
9	gi|2444180	unconventional myosin	IEKDFR	Helianthus annuus	39	MT leaf	MZM cup gall<<MZM leaf	Figure.7
10	gi|9757944	MAP kinase	DEEAAGQSMK	Arabidopsis thaliana	40	MT leaf	All both galls<<leaves	Figure 5-10

11	gi|3914592	RuBisCO small subunit	EVDYLLR/SPGYYDGR/IIGFDNKR	Betula pendula	135	MT leaf	All both galls<<leaves	Figure 5-10
12	gi|3914592	RuBisCO small subunit	EVDYLLR/SPGYYDGR/IIGFDNKR	Betula pendula	112	MT leaf	All both galls<<leaves	Figure 5-10
13	gi|50942283	lipid binding	NSASCTRGCR	Oryza sativa (japonica cultivar-group)	30	MZM cup gall	MZZ/MZM cup galls >> MZZ/MZM leaves,MZZ mouse gall, MT cup gall	Figure 5/7/12/15/16
14	gi|15293147	lipid binding	GSNGSSANRSR	Arabidopsis thaliana	33	MZM cup gall	MZZ/MZM all both galls>> MZZ/MZM leaves, MT both galls	Figure 5-8/15/16/18/19
15	gi|2244755	lipid binding	IAWDFR	Arabidopsis thaliana	39	MZM cup gall	MZZ/MZM cup galls>> MZZ/MZM leaves	Figure.5/7
16	gi|18403457	lipid binding	TTAVSLPR	Arabidopsis thaliana	29	MZM cup gall	MZZ/MZM all both galls>> MZZ/MZM leaves	Figure 5-8
17	gi|15233079	AHP1 (HISTIDINE-CONTAINING PHOSPHOTRANSMITTER 3)	NACVVFR	Arabidopsis thaliana	39	MZM cup gall	MZZ/MZM all both galls>> MZZ/MZM leaves, MT both galls	Figure 5-8/15/16/18/19
18	gi|50922561	OSJNBb0068N06.5	ASAIDPLAPAPNKK	Oryza sativa (japonica cultivar-group)	42	MZM cup gall	MZZ/MZM all both galls>> MZZ/MZM leaves, MT both galls	Figure 5-8/15/16/18/19
19	gi|9796478	serine threonine kinase homolog COK-4	LEICIGVAR	Phaseolus vulgaris	39	MZM cup gall	MZZ/MZM all both galls >>MZZ/MZM leaves	Figure 5-8
20	gi|77551957	putative pectin methylesterase	FLGGGGVDQDNRLK	Oryza sativa (japonica cultivar-group)	35	MZM cup gall	MZZ/MZM all both galls>> MZZ/MZM leaves, MT both galls	Figure 5-8/15/16/18/19
21	gi|18403457	lipid binding	TTAVSLPR	Arabidopsis thaliana	31	MZM cup gall	MZM cup gall>>MZM leaf	Figure.7
22	gi|50908617	lipid binding	KSGGGGSGEGSR	Oryza sativa (japonica cultivar-group)	38	MZM cup gall	MZM cup gall>>MZM leaf	Figure.7

23	gi|9796478	serine threonine kinase homolog COK-4	LEICIGVAR	Phaseolus vulgaris	45	MT leaf	All both galls<<leaves	Figure 5-10
24	gi|18403457	lipid binding	TTAVSLPR	Arabidopsis thaliana	32	MT cup gall	MT both galls>> MZM/MZZ galls	Figure15/16/18/19
25	gi|18403457	lipid binding	TTAVSLPR	Arabidopsis thaliana	30	MT cup gall	MT both galls>> MT Leaf, MZZ/MZM both galls	Figure.9/10/15/16/18/19
26	gi|24473812	small ribosomal subunit protein 4	TNSINQSIFNKK	Bryum argenteum	46	MT cup gall	MT both galls>> MZM,MZZ both galls	Figure.15/16/18/19
27	gi|7573598	protein kinase 2	DEISKDIR	Populus nigra	54	MT cup gall	MT both galls>> MZM/MZZ both galls	Figure.15/16/18/19
28	gi|18403457	lipid binding	TTAVSLPR	Arabidopsis thaliana	32	MZM leaf	All both galls<<leaves	Figure 5-10
29	gi|18403457	lipid binding	TTAVSLPR	Arabidopsis thaliana	31	MZM leaf	All both galls<<leaves	Figure 5-10
30	gi|464022	RuBisCO large subunit	XKESVGFK	Stylobasium australe	26	MT leaf	MT leaf>> MZM/MZZ leaves,MT both galls	Figure 5/7/9/10
31	gi|92872159	Tetratricopeptide-like helical	QVSESTLK	Medicago truncatula	23	MZM leaf	MZM both galls<<MZM leaf	Figure 7/8
32	gi|15225402	cyclin-dependent protein kinase	DSLAISPR	Arabidopsis thaliana	30	MZM leaf	MZM/MT both galls<< MZM/MT leaves	Figure7-10
33	gi|18403457	lipid binding	TTAVSLPR	Arabidopsis thaliana	30	MZM cup gall	MZM both galls<<MZM leaf	Figure 7/8
34	gi|15225402	cyclin-dependent protein kinase	DSLAISPR	Arabidopsis thaliana	33	MZM leaf	All both galls<<leaves	Figure 5-10

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