MOLECULAR IDENTIFICATION, PHYLOGENY AND SYSTEMATICS OF *HAEMOGREGARINA* IN TURTLES

THESIS

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By

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Molecular Identification, Phylogeny and Systematics of *Haemogregarina* in turtles

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For Mom

My biggest fan

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CHAPTER 1

HAEMOGREGARINA:

LIFE CYCLE, HOST SPECIFICITY,

PATHOGENICITY AND COEVOLUTION

Molecular analyses have proven invaluable tools for systematics, phylogenetics, and evolutionary biology. The comparison of multiple species gives information on the adaptive processes and evolutionary history, thus revealing the chronology of events. These evolutionary events can be mapped onto phylogenetic trees based on synapomorphies between taxa. There are multiple techniques available to construct phylogenetic trees based on morphological characters, molecular characters, other shared characters or combinations of these. By recreating evolutionary history based on these synapomorphic characters, the phylogenetic hypotheses seek to resolve questions of ancestry and descent. Phylogenetic reconstructions based on the morphology of primitive parasites, such as protozoa, prove difficult due to ambiguous terminology, functional speculation, determination of primitive or derived characters, and establishment of homology.

The phylum Apicomplexa includes unicellular eukaryotic pathogens that parasitize the entire range of organisms, from other protozoa to higher vertebrates (Kudo, 1966). Apicomplexans are in the kingdom Protista including unicellular algae and slime molds. Ellis et al. (1998) noted approximately 4600 species that have been described for Apicomplexa, and estimate undescribed members to include thousands more. They are characterized as "apical complex" due to the synapomorphic polar ring, rhoptries, micronemes, conoid, and subpellicar microtubules (Barnard & Upton, 1994). The mode of obtaining nutrition from the host includes dissolved cytoplasm, tissue fluid, body fluid, or dissolved nutrients that are absorbed by the parasite (Kudo, 1966). In general, Apicomplexans that parasitize reptiles are either blood or intestinal parasites. Apicomplexan blood parasites are transmitted by an intermediate host including ticks, leeches, and mosquitoes and intestinal parasites are transmitted by fecal contamination. Pathogenic genera include *Plasmodium* (infecting >300 M humans with malaria annually), *Eimeria* (parasitizing domesticated animals), *Toxoplasma* (the well-known disease agent that is transmitted by cats to humans and other animals), and *Cryptosporidium* (the gut parasite that inflicts diarrhea on humans and other vertebrates).

Haemogregarina

The family Haemogregarinidae (Protozoa: Apicomplexa: Coccidia) includes the genera *Hepatozoon, Haemogregarina, Cyrilia,* and *Karyolysus,* and recently included members are *Desseria* and *Hemolivia* (Smith et al., 2000). Members of this family are intraerythrocytic parasites in a wide range of vertebrates, including reptiles, amphibians,

fish, birds, and mammals. Hemogregarines are characterized by the asexual life cycle in a vertebrate circulatory system (intermediate host) and the sexual life cycle in the digestive system of a hematophagous invertebrate vector (definitive host). The genera are distinguished based on developmental and ultrastructural characteristics, range of hosts, and life cycle phases. Only two genera, *Hepatozoon* and *Haemogregarina*, are reported to parasitize chelonians. *Haemogregarina* is typically associated with turtles whereas *Hepatozoon* is typically associated with amphibians, snakes and lizards, crocodiles, and some mammals. Many species have been placed in the genus *Haemogregarina*, however only those utilizing a leech vector may actually belong to this genus (Smith, 1996). It is estimated that there are over 400 species of hemogregarines, (Desser, 1993) however there are inconsistencies in the phylogeny and taxonomy due to difficulty in identifying them morphologically.

Life cycle

Hemogregarines were first described by Russian Vasili Yakovlevich Danilivskii in 1885 who discovered the parasites in erythrocytes of the turtle *Emys orbicularis* and named it *Haemogregarina stepanowi* (Levine, 1988). Apicomplexan protozoans were first been described 200 years earlier by Antony van Leeuwenhoek (Levine, 1988). Hemogregarines are intraerythrocytic or interleukocytic parasites with a heteroxenous life cycle. This involves merogony in the circulatory system of the vertebrate and sporogony in the intestinal epithelium of the invertebrate vector (Barnard & Upton, 1994).

Haemogregarina includes more than 300 species (Barnard & Upton, 1994); however most hemogregarine genera are classified in the genus *Haemogregarina* until the life cycles are elucidated (Levine, 1982). It is expected that many of these species will be subsequently placed more correctly in the genus *Hepatozoon* (Allison & Desser, 1981) and only those using the leech vector included in the genus *Haemogregarina* (Smith, 1996). *Haemogregarina* is typically identified in vertebrate hosts by gamonts in peripheral blood erythrocytes, and in invertebrate vectors by oocysts in the intestine.

Reichenow (1910) described the life cycle, structure, and vector status of the leech, *Placobdella catenigera* in *Emys orbicularis,* the European pond turtle (Paterson & Desser, 1976). Michel (1973) established that species of *Haemogregarina* and *Hepatozoon* may occur in chelonians as he described *Hepatozoon mauritanicum* from *Testudo graeca* and the tick *Hyalomma aegyptium* (Paterson & Desser, 1976). As other researchers have contributed information on the infections of the various hosts, the practice of giving a new specific epithet to parasites of a different host has become common (Ball et al., 1967). This created much confusion, as accurate taxonomy can only be determined by cross-transmission studies and elucidation of the entire life cycle. However, there is much variability in the host specificity for both the vertebrates and invertebrates. For example, Ball et al. (1967) reported that a single species can live in a snake and a lizard, and be transmitted by ticks, mosquitoes, and mites; contrary to some parasites being specific to one host and vector (Levine, 1982). Other studies have shown a readily ability to infect some genera, and an inability to infect other genera (Ball, 1967).

If cross-transmission and entire life cycle studies are not performed, using *Haemogregarina sensu lato* was suggested by Mohammad & Mansour (1959).

Hemogregarines evolved a complicated heteroxenous life cycle that requires two hosts to complete. The parasites survive in the blood and other tissues of vertebrate hosts and in the hemocoel, gut epithelium, or reproductive tract of the definitive invertebrate hosts (Desser, 1993). The generalized life cycle involves three major components: merogony, gametogony, and sporogony. The infective merozoite passively enters the turtle host from the proboscis of the infected leech during blood feeding. Preerythrocytic merozoites encyst in the liver, lung, and spleen to produce more merozoites. The apical complex (hence, the phylum name Apicomplexa) is located at the anterior pole of this elongated invasive cell. Apical organelles secrete surface ligands necessary to attach to and invade the host cell, which positions the sporozoite in a parasitiphorous vacuole (Menard, 2001). This vacuole is in part derived from the plasma membrane of the host cell (Menard, 2001), thus prohibiting an antigenic response. These invasive merozoites are in the erythrocytes, liver, lung, and spleen of the vertebrate host (Siddall & Desser, 1991). Merozoites continue in either of two paths: some will continue several rounds of merogony (asexual reproduction) to produce more merozoites; and most will undergo gametogony to produce anisogamous gamonts. Infected blood cells are subsequently ingested by the leech host. Microgamonts and macrogamonts are released and undergo syzygy. These gametes fuse through sexual reproduction to produce a zygote. This zygote undergoes sporogony (asexual reproduction to produce sporozoites) thus

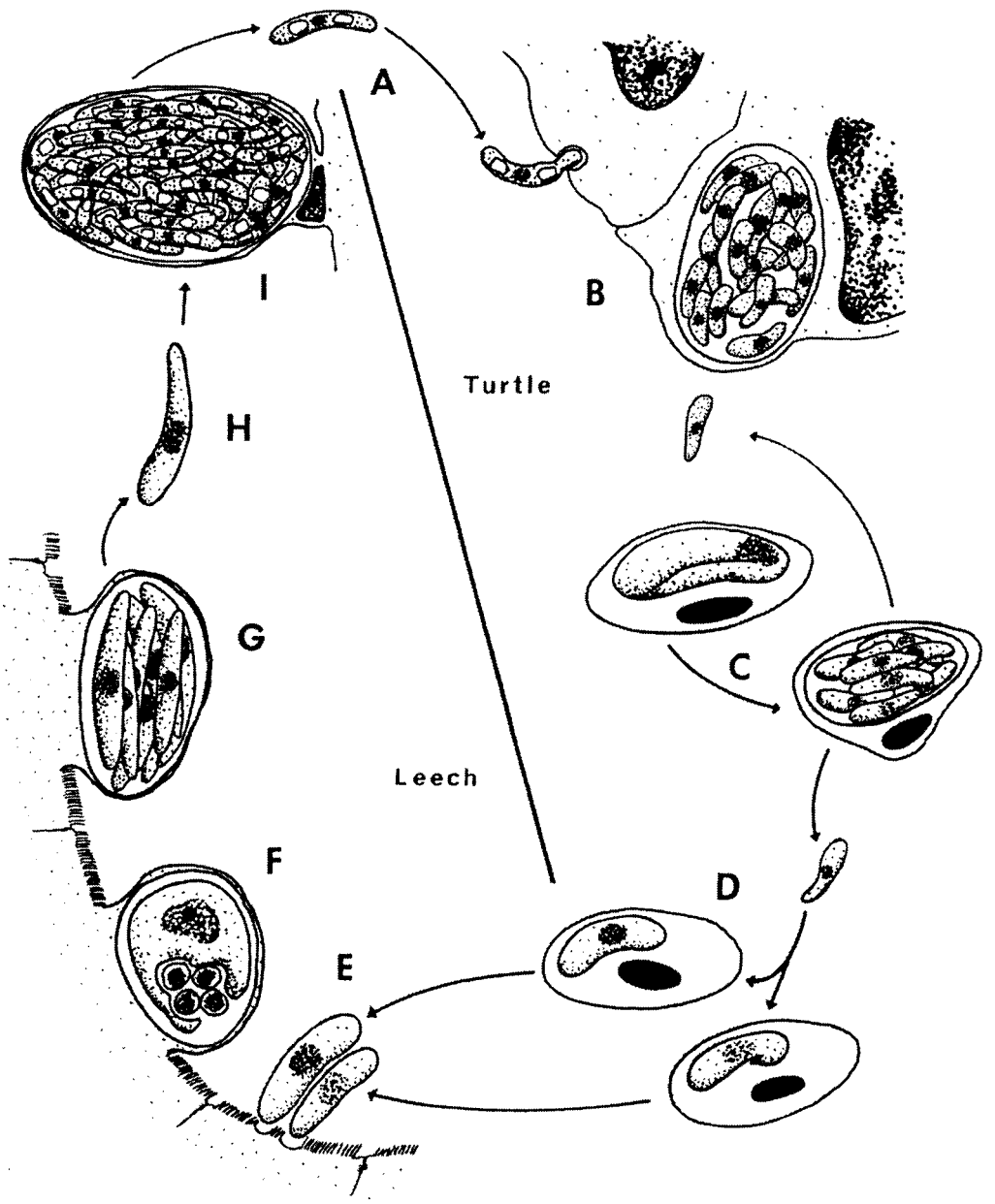


Figure 1. Schematic diagram of the life cycle of *Haemogregarina balli*. A. Infective merozoites are transmitted to turtles from the proboscis of leeches during feeding. B. Preerythrocytic meronts in cells of the liver, lung, and spleen yield about 18 merozoites. C. Immature serpentine erythrocytic meronts. The 8 merozoites formed in erythrocytic meronts infect other erythrocytes to produce either gamonts or more meronts. D. Microgamonts and macrogamonts in the peripheral circulation are ingested by leeches feeding on the turtle. E. Gamonts associate in syzygy in the intestinal ceca. F. Microgametogenesis generates 4 aflagellate microgametes, 1 of which fertilizes the associated macrogamete. G. Sporogony produces monosporoblastic oocysts with 8 sporozoites. H. Sporozoites migrate to the anterior somites of the leech, and give rise to primary meronts each containing hundreds of merozoites. Merozoites move to the proboscis of the leech until introduced to turtles during subsequent feedings. (Entire diagram and legend from Siddall & Desser, 1991).

completing the life cycle. Figure 1 shows the *Haemogregarina* life cycle proposed by Siddall & Desser, (1991).

Haemogregarina and *Hepatozoon* sp. have been reported in every family within Testudines (See Chapter 2, Table 2), however these are probably all appropriately placed in the genus *Haemogregarina* if vectored by a leech. It is not clear, however, what host specificity, if any, exists for these parasites within Reptilia. Consequently the transmission of the parasites is not clear, however experimental transfer studies have shown that an infected vertebrate host can transmit the blood parasite via an previously uninfected definitive vector to an previously uninfected vertebrate host.

Host specificity

Within the family Haemogregarinidae, the majority of the host specificity research has focused on the amphibian parasite *Hepatozoon*. Chao & Ball (1967) and Ball et al. (1967) reported a single species of *Hepatozoon* to infect three genera and two families of snakes however did not report any host morbidity. Kim et al. (1998) could readily infect *Hepatozoon clamatae* in *Rana* (frogs), however they were unsuccessful in infecting *Bufo* (toads) or *Ambystoma* (salamanders). This study also demonstrated that experimental infections of two species of hemogregarines *Hepatozoon clamatae* and *H. catesbianae* in *Rana clamitans melanota* (green frogs) and *Rana catesbeiana* (bullfrogs) show differences in the levels of infection in the respective vertebrate hosts. The authors

proposed that the different species of parasites were possibly more adapted to their respective ranid.

Siddall and Desser (1991) demonstrated the transmission of *Haemogregarina balli* from painted turtles to snapping turtles through the definitive leech host. Smith et al. (1996) reported the tendency of a low specificity of intermediate host (i.e., anuran) and a higher specificity to complete the life cycle in the second intermediate host (i.e., snake that preys on infected anuran) in an experimental transfer study. Telford et al. (2001) suggested a greater host specificity by extensively describing and naming six "new" *Hepatozoon* species in snakes, however this nomenclature is premature based on morphologic characters exclusively without elucidation of the life cycle.

Pathogenicity

It is generally considered that the parasite is non-pathogenic in its natural host, however can cause pathological effects when infected experimentally to un-natural (not normally occurring in) hosts (Wozniak et al., 1996). The longer a parasite and a host have a relationship, the parasite becomes more adapted to the host and less disruptive to the host's metabolism. Wozniak et al. (1996) described symptoms of infection in natural hosts as slight anemia, erythrocyte hypertrophy, and erythrocytic plasma membrane alterations. Other researchers reported anemia and inanition (Barnard & Upton, 1994), and distorted cell nuclei (Hahn, 1909). Beyer & Sidorenko (1972) detailed in a cytochemical examination of parasitized rock lizards a decrease in hemoglobin implying

a parasite-induced metabolic change in the host cell. They also demonstrated that the total protein quantity remained constant in infected individuals and suggested that the parasite's protein production was responsible. Oppliger et al. (1996) discovered that lizards parasitized by hemogregarines showed a decrease in oxygen consumption and lower locomotor speed.

In un-natural hosts, however, the symptoms may include increased inflammatory cells and clinical disease were reported in snakes (Wozniak et al., 1996). Wozniak et al. (1996) showed that lizards infected with snake hemogregarines produce antibodies detectable by ELISA assays. Hemogregarine infection was also shown to induce mortality in older hosts.

Lowichik and Yaeger (1987) reported congenital transmission in one brood of *Nerodia fasciata confluens* (water snake) in southeastern Louisiana. In this study, five gravid females from *Nerodia* and *Agkistrodon* were diagnosed with *Hepatozoon* infection. These females were removed from the Louisiana wild population and maintained in captivity. On the day of birth for the newborn snakes, blood smears were examined from the newborns and also from the female. One of the five gravid females transmitted the infection to 100% (n=12) of the offspring, and Lowichik and Yaeger (1987) attributed this to the higher parasitemia of this female. The other four gravid females with lower level parasitemia did not transmit the parasite to their offspring.

In an experimental transfer study by Oda et al. (1971), researchers successfully infected snakes and lizards by feeding them mosquitoes or infected organs from an infected vertebrate for two species of *Hepatozoon*. Interestingly, the range of infection lasted from 7 days in a *Sceloporus* (lizard) to 1.5 years in a *Pituophis* (snake). They concluded that the hemogregarines show versatility in infecting un-natural hosts. Furthermore, studies involving experimental transfer often are successful with certain hosts and unsuccessful with others (Oda et al., 1971; Kim et al., 1998).

The pathogenicity of hemogregarines remains uncertain in general and must be considered on an individual basis. Oppliger & Clobert (1997) examined lizard tail regeneration in *Lacerta vivipara* for individuals infected and individuals not infected with hemogregarines. They concluded that parasitized lizards had a drastically reduced rate in tail regeneration, however parasitemia did not increase in association with the tail loss. In a similar study, the same researchers determined that *Haemogregarina*-infected lizards have lower levels of hemoglobin, increased immature erythrocytes, decreased resting oxygen consumption, and a reduced running velocity (Oppliger et al., 1996). Sorci (1996) found that in the same common lizard (*Lacerta*), that hemogregarines possibly have a considerable age-dependent effect on host mortality and induce mortality only in older hosts. Wozniak et al. (1996) showed that lizards infected with snake hemogregarines have a humoral response with common and stage-specific components. They suggest that this inflammatory reaction is in response to pathogenesis in the un-natural host species. Wozniak and Telford (1991) also demonstrate that hemogregarines increase mortality and morbidity rates in the mosquito vector *Culex*.

Ecology

When considering the ecological environment of the hemogregarine parasite, it is necessary to evaluate the turtle circulatory system as an ecological environment. The mean duration of turtle erythrocytes is between 600 and 800 days, indicative of the low metabolic rate and hibernation that is characteristic of reptiles (Altland & Brace, 1962). Therefore, initial parasite infection may last several years. Other studies showed that aspects including sex, age, diet, health, exercise, circulating hormones, hydration of body, temperature, and oxygen pressure can effect the turtle packed erythrocyte volumes (PCV) (Frair, 1977). Frair (1977) also reported that red blood cell counts (RCC) tends to increase with age, that larger organisms tend to have higher PCV, and seasonal increase in erythropoiesis in summer. Turtles maintained in captivity were proposed to have significantly higher blood chemistries as a result of artificial diets or stressful conditions (Bolten, 1992). Frair (1977) stated that wild turtles tend to have higher PCV and RCC levels compared to turtles in captivity.

Captive management may increase the susceptibility of exposure to wildlife diseases, including *Haemogregarina*. Captive husbandry for turtles often involves multiple genera housed collectively and this may lead to trans-generic infections. Certain wildlife diseases, therefore, may cause severe antigenic responses in hosts not previously exposed (unnatural populations). In an effort to reduce the potential of infection in captive managed populations, it is essential to elucidate the entire range of epidemiological factors.

Epidemiology & Distribution

The factors affecting the epidemiology and distribution of hemogregarine interactions include vector exposure, host specificity, and pathogenicity. The current problems in these studies involve inadequate evaluation of the entire life cycle to determine the appropriate hemogregarine and vector involved, novel species names for different hosts, limited cross-transfer studies due to extensive time involved, and inadequate descriptions of pathological effects, if any, on the hosts. Without extensive phylogenetic studies of the hemogregarines, host specificity questions could be lengthy and time consuming.

Coevolution in host-parasite systems

When evaluating host-parasite associations, reconstructing evolutionary history traces to one of two paths. The first path is one which the parasites coevolved (or co-speciated) simultaneously with the host. This occurs as the speciation of the host group initiates a subsequent speciation of the associated group (Huelsenbeck et al., 1997). Congruence between parasite and host phylogenies would show this coevolutionary result. The second path is one which the parasite does not necessarily coevolve with the host, however other associations can be determined. Phylogenies reveal that hosts which are phylogenetically close share common parasites because of a shared recent evolutionary history (Poulin & Morand, 2000). It is necessary to determine the phylogenies for both host and parasite to determine the two evolutionary histories and

then to explore coevolutionary hypotheses (Poulin & Morand, 2000). By investigating the patterns of evolutionary history, one can thereby investigate the processes enforcing these patterns.

Brooks (1979) terms coevolution as "the mutual adaptation of a given parasite species and its host(s) through time and includes such parameters as pathogenicity, host specificity, and synchrony of life cycle stages." There are certain associations that are ideal for coevolutionary studies including host groups as well as parasite groups that are species rich, geographically widespread, and ancient in origin (Brooks & McLennan, 1993).

It has been assumed by parasitologists that parasites lack the ability to choose different environments and therefore evolutionarily dependent upon their hosts (Brooks, 1979). This attributes to the phylogenies and ecological distributions of the parasites being closely interrelated to their hosts. Based on these premises, the following standards (excerpt from Brooks & McLennan, 1993) for deducing coevolutionary contexts in host-parasite relationships were formed:

1. **Farenholz' Rule**: *The historical development (anagenesis) and splitting (cladogenesis) of hosts are paralleled by the development and splitting of their parasites. The phylogenetic relationships of the parasites can therefore be used to draw conclusions about the (often obscured) phylogenetic relationships of their hosts.*

2. **Szidat's Rule**: *The orthogenetic trend toward higher development in hosts drives their (mainly permanent) parasites in the same direction. Because of this, host taxa with a relatively low level of organization (primitive hosts) harbor parasites with relatively low levels of organization (primitive parasites).* He further stated that non-specific parasites had main and secondary hosts, and that such nonspecificity was indicative of younger parasites (i.e., younger parasites tend to have more secondary hosts), because as Szidat's rule demonstrated, host specificity tends to increase with increasing age of the parasite-host association.

3. **Eichler's Rule**: *Among host groups of equal systematic rank, species-rich groups will have a more diverse (mainly permanent) parasite fauna than their species-poor relatives.*

4. **Manter's Rules**:
    a. *Parasites evolve more slowly than their hosts.*
    b. *The longer the association with a host group, the more pronounced the specificity exhibited by the parasite group.*
    c. *A host species harbors the largest number of parasite species in the area where it has resided the longest, so if the same species, or two closely related species of hosts, exhibit a disjunct distribution and possess similar faunas, the areas in which the hosts occur must have been contiguous in the past.*

Previous host-parasite associations in coevolutionary studies involve protozoa due to their evolutionarily conservative nature required to meet both vertebrate and invertebrate host conditions (Ayala & Hutchings, 1974). Zoogeographical studies within Reptilia include *Plasmodium* in lizards (Ayala & Hutchings, 1974; Perkins, 2001), and *Sarcocystis* in snakes (Dolezel et al., 1999). There have not been any coevolutionary studies with apicomplexans in turtles, however, blood flukes in turtles have been studied to explain continental drift (Brooks & McLennan, 1993).

In tracing the epidemiology and coevolution of hemogregarines, proper identification is foremost. This can be accomplished morphologically by examination of peripheral blood for the intraerythrocytic parasites, and recently by molecular methods of Polymerase Chain Reaction with parasite-specific primers. This information impacts both wild populations and captive managed populations to reduce any threatening wildlife disease that pose risks to not just a single individual but to an entire population.

The widely-distributed reports of the occurrence of *Haemogregarina* in turtles prompted the investigation of all accessible populations for the current study. This included endemic Texas turtle genera from several localities as well as translocated Asian turtles under captive management. Translocation of turtles can spread disease therefore the initial step is identification and quantification of parasitemia within each turtle subpopulations. In an effort to evaluate the potential infection of *Haemogregarina* in

naturally-occurring populations and translocation populations, the following objectives were delineated:

1) Determine parasitemia in captive, wild, and recently translocated turtles from several localities;

2) Compare diagnosis of parasitemia using both microscopy and molecular (PCR) techniques and evaluate the efficacy of each respective technique;

3) Make comparisons among host subpopulations to determine trends in epidemiology and transmission to evaluate parasite control;

4) Determine molecular phylogeny of the phylum Apicomplexa and the placement of *Haemogregarina* genus within Apicomplexa;

5) Examine the molecular phylogeny of *Haemogregarina* to determine host specificity and coevolutionary status for parasites at various genera turtle localities; and,

6) Determine methods to control the risk of exposure and transmission of *Haemogregarina* for captive managed turtles.

CHAPTER II

DETECTION AND DIAGNOSIS OF *HAEMOGREGARINA*: MORPHOLOGICAL VERSUS MOLECULAR IDENTIFICATION

Prior to the advent of recent molecular techniques, apicomplexans were identified and classified solely on their morphological characters and life history. This included size and shape of various life cycle stages and the location of infection. Morphology, vertebrate host, invertebrate host, ecological and biogeographical conditions were all utilized to produce phylogenies. This proves problematic, however, due to ambiguous terminology, functional speculation, determination of primitive or derived characters, and in establishing homology. Today both morphology and molecular data are used in identification, systematic classification and phylogenetic reconstructions.

Morphologic Detection

Diagnosis of haemogregarine infection by light microscopy provides both qualitative and quantitative results. Requiring few tools and little training, stained blood smears can be diagnosed in the field providing immediate results. Multiple infections can

be diagnosed, however the epidemiology of infections proves more difficult to survey. The limitations of this technique include ambiguous positive or negative results, as low parasitemia infections might not be detected on a single smear, and difficulty in accurate genera determination using morphology exclusively. When considering primitive protozoa, there are very limited morphological characters for descriptions and these characters may be the result of homoplastic convergence.

Molecular Detection

While molecular techniques only provide qualitative results, there are more explicit and definable characters (nucleotides) that may be used to determine homology. The benefits of the molecular sequence characters over morphologic characters include obtaining accurate species identification, no *a priori* concept of primitive or derived characters, and no ambiguous descriptive terms as often found in apicomplexan morphologic characters. Molecular data are not a panacea, however, as the limitations of only four character states (A, C, G, and T) can also lead to homoplasy from convergence and back substitutions.

For identification and phylogenetic analyses in the Apicomplexa, ribosomal DNA genes including 18S, 5.8S, and ITS regions have been used (Cai et. al, 1992; Goggin, 1994; Kim et al., 1998; Mathew et al, 2000; Moon-van der Staay, 2001; Perkins & Keller, 2001; Smith et al., 1999; and Wozniak & McLaughlin, 1993). These genes can be used to determine morphologically indistinguishable but otherwise distinct species (Hillis &

Dixon, 1991). The small subunit ribosomal DNA from the apicoplast genome has also proven to resolve taxonomic differences and phylogenies (Obornik et al., 2002). Figure 2 shows a diagram of the ribosomal DNA gene family in eukaryotes (Hillis & Davis, 1986).

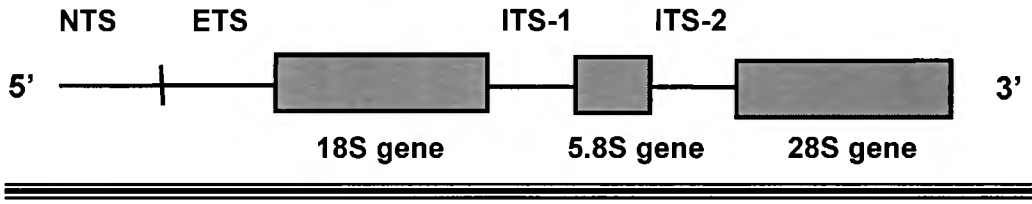


Figure 2. Diagram of the ribosomal DNA gene family in eukaryotes (Hillis & Davis, 1986). The shaded blocks show the 18S, 5.8S, and 28S genes coding regions. The NTS is the nontranscribed spacer, the ETS is the external transcribed spacer, and the ITS regions are the internal transcribed spacer regions. In eukaryotes, the 18S = 1800 nt, 5.8S = 160 nt, and 28S = over 4000 nt.

The small subunit of ribosomal DNA is often employed to determine phylogenetic relationships of eukaryotes. This molecule's attributes include its conservation due to functional constraints, variable mutation rates in different substructure positions, and its ubiquity among all taxa (Ouvrard et al., 2000). The 18S subunit contains a region of low substitution rates as well as regions of high substitution rates. This allows for phylogenetic resolution of organisms of deep divergence (between classes) as well as recent divergence (between species). The approximate length in eukaryotes for the ribosomal genes are 18S = 1800 nt, 5.8S = 160 nt, and 28S = over 4000 nt. (Hillis & Dixon, 1991). There are two internal transcribed spacers: one between

18S and 5.8S and the other between 5.8S and 28S. The transcribed spacers contain signals for processing the rRNA transcript (Hillis & Dixon, 1991).

Table 1 shows a comparison of morphological to molecular methods for identification of *Haemogregarina*. The benefits and limitations of each method are outlined to provide an overview. The traditional method of identification using parasite morphology has been used for over 100 years, whereas the molecular techniques for identification and systematics of apicomplexans have only been used for the past 10 years.

Table 1. Comparison of morphological to molecular methods for identification of *Haemogregarina*. The benefits and limitations of each method are outlined as an overview.

Morphology	Molecular
Immediate results (in the field)	Requires 24 hrs. for results (in the lab)
Inexpensive and few tools	Costs $70 -$200 per sample to extract & PCR
Qualitative and quantitative results	Only qualitative results
Little training required	More training required
Ambiguous slides as pos/neg	PCR test is explicit as positive or negative
Difficulty in identifying genera	Sequences are accurate genera ID
Must make blood smears on site	Can use stored blood or if no slides available
Can not determine epidemiology	Can track the spread of disease
Check for other infections	Specific primers diagnose specific genera

In tracing the epidemiology for any disease, proper identification is essential. Morphologically indistinguishable parasites may potentially represent different genera. In an effort to control disease, accurate identification is the first step in understanding transmission, pathogenicity, and disease control measures. Wildlife diseases can have

potentially devastating effects, particularly in small populations or those involving endangered species. Certain wildlife diseases are observed in only captive populations, only wild populations, or in both sets of populations. *Haemogregarina* infections occur in an almost worldwide distribution in a wide range of hosts. The effect of wild populations versus captive managed populations is of interest to animal husbandry, especially in regards to translocated animals.

The worldwide increasing species extinction rates in turtle populations is attributed to land encroachment, urbanization, pollution, habitat destruction, and commercial harvesting. This worldwide phenomenon is affecting extremely abundant taxa as hastily and indiscriminating as it affects endangered taxa. Turtles are particularly susceptible to escalating extinction rates due to delayed sexual maturity, high juvenile mortality, and long adult life-span with low natural mortality. Due to the Asian Turtle Crisis in which endangered species are harvested for decorative shells and believed medicinal benefits in the meat and ground shell, the threat of extinction is now a reality. The multimillion dollar trade in turtles in the Asian region has led to a biodiversity crisis with 75% of species at risk of extinction. This has led to both *in situ* and *ex situ* conservation strategies. *Ex situ* programs may potentially create infections in unnatural hosts and thus decrease the viability of *ex situ* or captive populations.

Translocation is far from an ideal situation, however is often times crucial in the perpetuation of species for future reintroduction into a natural environment. *Geochelone yniphora*, the angulated tortoise, was designated as endangered in its only range in

Madagascar in 1976. This tortoise is virtually extinct in the wild today, and persists exclusively due to captive management. The reintroduction of the wolf in Yellowstone National Park is yet another example of an extirpated species that was perpetuated for reintroduction. These *in situ* and *ex situ* conservation strategies often result in mixed populations of turtles which may increase the incidence of exposure to wildlife diseases. The occurrence of parasites, such as *Haemogregarina*, in these populations may be overlooked and therefore the factors affecting the epidemiology not understood.

Affected turtles

Table 2 lists naturally-occurring haemogregarines previously recorded in turtles. Additional studies involving experimental transfer from natural hosts to unnatural hosts are not included. This table shows that *Haemogregarina* and *Hepatozoon* collectively have been reported worldwide in every family of turtles.

The reports on the wide distribution of *Haemogregarina* in turtles prompted the investigation of all accessible populations for the current study. This includes endemic Texas turtle genera from several localities as well as translocated Asian turtles under captive management.

Table 2 List of naturally-occurring haemogregarines previously recorded in turtles This does not include experimental transfer infected individuals This table shows that *Haemogregarina* and *Hepatozoon* collectively have been reported worldwide in every family of turtles

TURTLE	HAEMOGREGARINE	REFERENCE
Amyda mutica	*Haemogregarina stepanowi*	Hahn, 1909
Aromochelys odoratus	*Haemogregarina stepanowi*	Hahn, 1909
Chelodina spp	*Haemogregarina chelodinae*	Johnston, 1909
Chelodina spp	*Haemogregarina chelodinae*	Laveran & Petit, 1909
Chelopus guttatus	*Haemogregarina stepanowi*	Hahn, 1909
Chelopus insculptus	*Haemogregarina stepanowi*	Hahn, 1909
Chelydra serpentina	*Haemogregarina* sp	Caskey, 1998
Chelydra serpentina	*Haemogregarina* sp	Desser, 1972
Chelydra serpentina	*Haemogregarina stepanowi*	Hahn, 1909
Chelydra serpentina	*Haemogregarina balli*	Paterson & Desser, 1976
Chelydra serpentina	*Haemogregarina stepanowi*	Roudabush & Coatney, 1937
Chelydra serpentina	*Haemogregarina balli*	Siddall & Desser, 1991
Chelydra serpentina serpentina	*Haemogregarina pseudemydis*	Acholonu, 1974
Chelydra serpentina serpentina	*Haemogregarina stepanowi*	Marquardt, 1966
Chelydra serpentina serpentina	*Haemogregarina nicoriae*	Wang & Hopkins, 1965
Chinemys reevesi	*Haemogregarina botuliformis*	Chai & Chen, 1990
Chinemys reevesi	*Haemogregarina chinemydis*	Chai & Chen, 1990
Chrysemys belli marginata	*Haemogregarina stepanowi*	Roudabush & Coatney, 1937
Chrysemys picta	*Haemogregarina stepanowi*	Hahn, 1909
Chrysemys picta	*Haemogregarina balli*	Paterson & Desser, 1976
Chrysemys picta marginata	*Haemogregarina balli*	Siddall & Desser, 1991
Chrysemys sp	*Haemogregarina stepanowi*	Plimmer, 1913
Cinosternum cruentatum	*Haemogregarina stepanowi*	Plimmer, 1913
Clemmys caspica	*Haemogregarina pelusiensi*	Pienarr, 1962
Clemmys elegans	*Haemogregarina labbei*	Borner, 1901
Clemmys insculpta	*Haemogregarina balli*	Paterson & Desser, 1976
Clemmys insculpta	*Haemogregarina balli*	Siddall & Desser, 1991
Clemmys japonica	*Haemogregarina clemmydis*	von Prowazek, 1909
Cuora flavomarginata	*Haemogregarina cuorae*	Chai & Chen, 1990
Cycloderma aubryi	*Haemogregarina reichenowi*	Schubotz, 1913
Damonia spp	*Haemogregarina stepanowiana*	Laveran & Mesnil, 1902
Emyda japonica	*Haemogregarina clemmydis*	von Prowazek, 1909
Emys leprosa	*Haemogregarina bagensis*	Ducloux, 1904
Emys lutaria (orbicularis)	*Haemogregarina stepanowi*	Danilewski, 1885
Emys meleagris	*Haemogregarina stepanowi*	Hahn, 1909
Emys orbicularis	*Haemogregarina nicoriae*	Reichenow, 1910
Graptemys kohni	*Haemogregarina pseudemydis*	Acholonu, 1974

Table 2 continued List of naturally-occurring haemogregarines previously recorded in turtles This does not include experimental transfer infected individuals This table shows that *Haemogregarina* and *Hepatozoon* collectively have been reported worldwide in every family of turtles

TURTLE	HAEMOGREGARINE	REFERENCE
Hydromedusa tactifera	*Haemogregarina hydromedusae*	Carini, 1942
Kinixys belliana zuluensis	*Haemogregarina fitzsimmonsi*	Santos Dias, 1953
Kinosternon pennsylvanica	*Haemogregarina stepanowi*	Hahn, 1909
Kinosternon subrubrum hippocrepis	*Haemogregarina pseudemydis*	Acholonu, 1974
Kinosternon subrubrum hippocrepis	*Haemogregarina nicoriae*	Wang & Hopkins, 1965
Malaclemys geographica	*Haemogregarina stepanowi*	Hahn, 1909
Mauremys capsica	*Haemogregarina* sp	Paperna, 1989
Nicoria trijuga	*Haemogregarina nicoriae*	Castellani & Wiley, 1904
Pelusios sinuatus	*Haemogregarina pelusiensi*	Paperna, 1989
Pelusios sinuatus	*Haemogregarina pelusiensi*	Pienaar, 1962
Pelusios sinuatus zulensis	*Haemogregarina maputensis*	Santos Dias & de Sousa, 1952
Pelusios sp	*Haemogregarina sternotheri*	Franca, 1912
Platemys sp	*Haemogregarina labbei*	Borner, 1901
Pseudemys concinna heriogiyphica	*Haemogregarina stepanowi*	Marquardt, 1966
Pseudemys floridana hoyi	*Haemogregarina pseudemydis*	Acholonu, 1974
Pseudemys floridana mobilensis	*Haemogregarina nicoriae*	Wang & Hopkins, 1965
Pseudemys floridana texana	*Haemogregarina nicoriae*	Wang & Hopkins, 1965
Pseudemys nelsoni	*Haemogregarina* sp	Caskey, 1998
Pseudemys scripta	*Haemogregarina stepanowi*	Hahn, 1909
Pseudemys scripta elegans	*Haemogregarina pseudemydis*	Acholonu, 1974
Pseudemys scripta elegans	*Haemogregarina stepanowi*	Marquardt, 1966
Pseudemys scripta elegans	*Haemogregarina stepanowi*	Wang & Hopkins, 1965
Pseudemys texana	*Haemogregarina* sp	Caskey, 1998
Pseudemys troosti	*Haemogregarina stepanowi*	Hahn, 1909
Sternothaerus adansonii	*Haemogregarina nicoriae*	Robertson, 1910
Sternothaerus adansonii	*Haemogregarina nicoriae*	Robertson, 1910
Sternotherus carinatus carinatus	*Haemogregarina nicoriae*	Wang & Hopkins, 1965
Sternotherus odoratus	*Haemogregarina stepanowi*	Marquardt, 1966
Terrapene carolina carolina	*Haemogregarina pseudemydis*	Acholonu, 1974
Terrapene carolina triunguis	*Haemogregarina pseudemydis*	Acholonu, 1974
Testudo emys	*Haemogregarina testudinis*	Laveran & Nattan-Larrier, 1912
Testudo graeca	*Hepatozoon mauritanicum*	Michel, 1973
Testudo graeca	*Haemogregarina ibera*	Tartakovskii, 1913
Testudo tabulata	*Haemogregarina dimorphon*	Brimont, 1909
Trachemys scripta	*Haemogregarina sp*	Caskey, 1998

Table 2 continued List of naturally-occurring haemogregarines previously recorded in turtles This does not include experimental transfer infected individuals This table shows that *Haemogregarina* and *Hepatozoon* collectively have been reported worldwide in every family of turtles

TURTLE	HAEMOGREGARINE	REFERENCE
Trionyx ferox emoryi	*Haemogregarina nicoriae*	Wang & Hopkins, 1965
Trionyx gangeticus	*Haemogregarina bongaonensis*	Sinha, 1993
Trionyx muticus	*Haemogregarina nicoriae*	Wang & Hopkins, 1965
Trionyx sinensis	*Haemogregarina galeata*	Chai & Chen, 1990
Trionyx sinensis	*Haemogregarina hubeiensis*	Chai & Chen, 1990
Trionyx sinensis	*Haemogregarina sinensis*	Chai & Chen, 1990
Trionyx spinifer	*Haemogregarina pseudemydis*	Acholonu, 1974

Two previous studies examined the haemogregarine parasitemia in Texas and provided a motive for investigating the infection. It was expected to find similar haemogregarine infection density in similar genera from previous studies. Thesis research completed by Caskey (1998) revealed infection rates in turtles from Spring Lake in *Trachemys scripta* (13/15), *Pseudemys texana* (22/31), *Pseudemys nelsoni* (2/2), and *Chelydra serpentina* (4/4). This research also described the levels of parasitemia ranging from light, moderate, to heavy parasitemia. Caskey (1998) then reported infection rates from the San Marcos River for *Pseudemys texana* (14/15), the Blanco River for *Pseudemys texana* (12/14), the Guadalupe River for *Pseudemys texana* (8/10), and Southwest Texas State University campus ponds for *Trachemys scripta* (3/6). Caskey (1998) did not observe schizonts in peripheral blood, but did discover schizonts with tiny merozoites in leukocytes. Caskey identified all parasites as *Haemogregarina* sp.

In another regional study of endemic Texas genera, Wang & Hopkins (1965) examined freshwater and terrestrial chelonians near College Station, Brazos County, Texas. Blood parasites were not found in any of the terrestrial chelonians examined: *Terrapene ornata, T. carolina triunguis,* or *Gopherus berlandieri* (specimen from Brownsville, Texas.) They reported infection rates in *Pseudemys* (=*Trachemys*) *scripta elegans* (15/19), *Kinosternon subrubrum hippocrepis* (2/2), *Sternotherus carinatus carinatus* (2/2), *Chelydra serpentina serpentina* (6/6), *Trionyx ferox emoryi* (1/1), *Trionyx muticus* (4/4), *Pseudemys floridana mobilensis* (=*P.concinna*) (1/1 from Port Arthur however misidentified because this species does not occur west of Mobile Bay, Alabama as per Dr. Francis Rose), and *Pseudemys texana* (2/2 from Austin). Wang & Hopkins (1965) considered descriptions for *Haemogregarina nicoriae* to fit most specimens infecting turtles in Texas.

The reports of the occurrence of *Haemogregarina* in turtles incited the investigation of all available populations for the present study including endemic Texas turtle genera from several localities as well as translocated Asian turtles under captive management. Translocation of turtles can spread disease therefore the initial step is identification and quantification of parasitemia within turtle subpopulations. In an effort to evaluate the potential infection of *Haemogregarina* in naturally-occurring populations and translocation populations, the following objectives were delineated:

1) Determine parasitemia in captive, wild, and recent captive turtles from several localities,

2) Compare diagnosis of parasitemia using both microscopy and molecular (PCR) techniques and evaluate the efficacy of each respective technique,

3) Make comparisons among host subpopulations to determine trends in epidemiology, transmission, and parasite control.

METHODS

Adult and juvenile turtles (see Appendix A) were collected by basking traps, hoop nets, or collected from captive ponds during the spring and summer of 2002. The IAUCC permit number is 7E1EC3_02 under Dr. Michael R.J. Forstner at Southwest Texas State University. Each turtle was categorized as "captive" if its entire life span remained in captivity. Turtles which had been relocated (primarily from Asia) within the past year were considered from "wild" populations. Turtles which were relocated from the wild but had spent more than one year in captivity were considered "wild caught, captive raised" (WCCR). Table 3 lists the localities from which turtles were collected for all further analyses. Captive populations included both indoor and outdoor facilities, and wild populations involved a variety of aquatic ecological habitats. See Appendix A for specific individuals for each locality.

Table 3. Overview of turtle collection localities for captive and wild populations. Captive populations included both indoor and outdoor environments, and wild populations involved a variety of ecological habitats.

Captive Populations	
Waterlife	Austin, Texas
Guthrie Turtle Farms	Birmingham, Alabama
Concordia Turtle Farm	Wildsville, Louisiana
Wild Populations	
Aquarena Springs	San Marcos, Texas
Private ranch	Deanville, Texas
Oasis Ranch	Sheffield, Texas
Capital Aggregate	Marble Falls, Texas
Griffith League Ranch	Bastrop, Texas

Turtles collected from Waterlife (Austin, Texas) and Guthrie Turtle Farm (Birmingham, Alabama) were housed in 3,000 gallon (= 11,360 L) pond aquaria maintained at 78-82 F (= 26-28 C) temperature. Multiple genera were housed within a single pond. Most of these individuals were not native and had been imported to the U.S. from various regions across Southeast Asia, primarily Malaysia and Indonesia. Turtles collected from Concordia Turtle Farm in Wildsville, Louisiana were maintained in outdoor concrete ponds at high density populations and were captured using sardine-baited hoop nets. All three localities have historically treated the pond water with copper sulfate as a control method for leeches.

Turtles were collected in basking traps from Cypress Point at Spring Lake at Aquarena Springs (San Marcos, Texas). Turtles collected from Deanville, Texas were captured in hoop nets baited with sardines on private property in a four acre isolated pond

surrounded with dense vegetation. Turtles collected at Oasis Ranch (Sheffield, Texas) from the Pecos River and local ponds were also collected from sardine-baited hoop nets. Turtles collected from Capital Aggregate located in Marble Falls, Texas were hand caught from the limestone basin creeks. The single individual collected from Griffith League Ranch located in the Lost Pines of Bastrop, Texas was discovered in a mud puddle.

Table 4. Taxonomic list of turtles, number of individuals, and region of turtle origin for individuals examined in this study.

Family	Included genera	Individuals	Region of origin
Chelidae (Austro-American side-neck turtles)	*Chelodina*	n=8	Papua New Guinea
	Elseya	n=11	Papua New Guinea
	Emydura	n=5	Irian Jaya, Indonesia, Papua
	Phrynops	n=6	Northern South America
	Platemys	n=2	Northern South America
Bataguridae (pond turtles)	*Callagur*	n=7	Borneo, Indonesia
	Cyclemys	n=4	E. India, S. China, Indonesia
	Geoemyda	n=1	S. China to Indochina
	Hardella	n=2	India
	Heosemys	n=20	Malaysia
	Hieremys	n=2	Vietnam to Thailand
	Orlitia	n=11	Borneo, Indonesia
	Rhinoclemys	n=1	Venezuela to Brazil
	Sacalia	n=2	Southeastern China
	Siebenrockiella	n=8	Sumatra, Indonesia
Emydinae (pond turtles)	*Chrysemys*	n=1	North America
	Graptemys	n=5	Central Texas
	Malaclemys	n=4	E. and S.E. coastline of U.S.
	Pseudemys	n=48	Eastern U.S. & NE Mexico
	Trachemys	n=180	NE Mexico to Central U.S.
Kinosternidae (mud & musk turtles)	*Kinosternon*	n=3	Southwest U.S. & Mexico
	Staurotypus	n=7	Central Mexico
	Sternotherus	n=90	North Americas & Mexico
Pelomedusidae (Afro-American side-necked turtles)	*Pelusios*	n=3	East & Southeast Africa
Trionychidae (softshell turtles)	*Apalone* (*Trionyx*)	n=1	North America

Morphological Identification

All turtles were marked, weighed, sex determined, and body measurements determined. Any external parasites (leeches) were noted for individuals, and several leeches were preserved in ethanol for future identification. Turtles were bled with a 20G needle from the femoral artery or vein. Thin blood smears were made following CDC protocol (http://www.dpd.cdc.gov Diagnostic Procedures for Blood Specimens) and in triplicate for each individual to ensure accuracy. Slides were fixed in absolute methanol for one minute, stained with Wrights Stain (Carolina Biological Supply) for three minutes, and placed in Wrights Buffer (Carolina Biological Supply) for six minutes following enclosed protocol using glass Coplin staining jars. After blood smears were made, all remaining blood per individual was mixed with 700 µl blood storage buffer (Appendix B) in Nunc® cryotubes. The cryotubes were labeled and stored at -80C as vouchers in the Forstner tissue collection at Southwest Texas State University. All molecular analyses were performed from this tissue. Initially the turtles were given a collector number (GLL number), and later a voucher numbers (MF number) for the permanent tissue collection.

Blood smears were subsequently examined for 10,000 cells for all three slides per individual. The level of parasitemia was determined by the number of infected cells per number of total cells. Intracellular parasites were measured in micrometers for genera identification. Any other distinguishable morphological characteristics and cytopathological effects on the turtle cell were noted.

Populations were compared using 95% confidence intervals between two groups. The following equation provided the method for analyses:

$$p_1\text{-}p_2 \pm \left[1.96^* \sqrt{\frac{(p_1(1\text{-}p_1)}{n_1} + \frac{p_2(1\text{-}p_2)}{n_2}} \right]$$

where p_1 is the proportion of infected individuals for the first sub-population evaluated, and p_2 is the proportion of infected individuals for the second sub-population. The n_1 and n_2 values are the total number of individuals for each sub-population respectively. All analyses were executed in Excel (Microsoft XP) using this formula.

Molecular identification

For alignment of sequences and development of primers, see chapter 3. Figure 3 shows an overview of the molecular methods used to isolate and amplify *Haemogregarina* sp. from turtle blood. The parasite DNA was isolated, amplified by PCR, and sequenced. A molecular method for identification, a visual band amplified by PCR, was performed to test the efficacy of the microscopy method of identification.

The Qiagen Dneasy® Extraction kit (#69506) and protocols for animal tissue were used to isolate both turtle and potential parasite DNA from blood stored in blood storage buffer. Initially, the primers from Smith et al. (1999) and protocols were attempted, however it was deemed necessary to develop novel primers (See Appendix D).

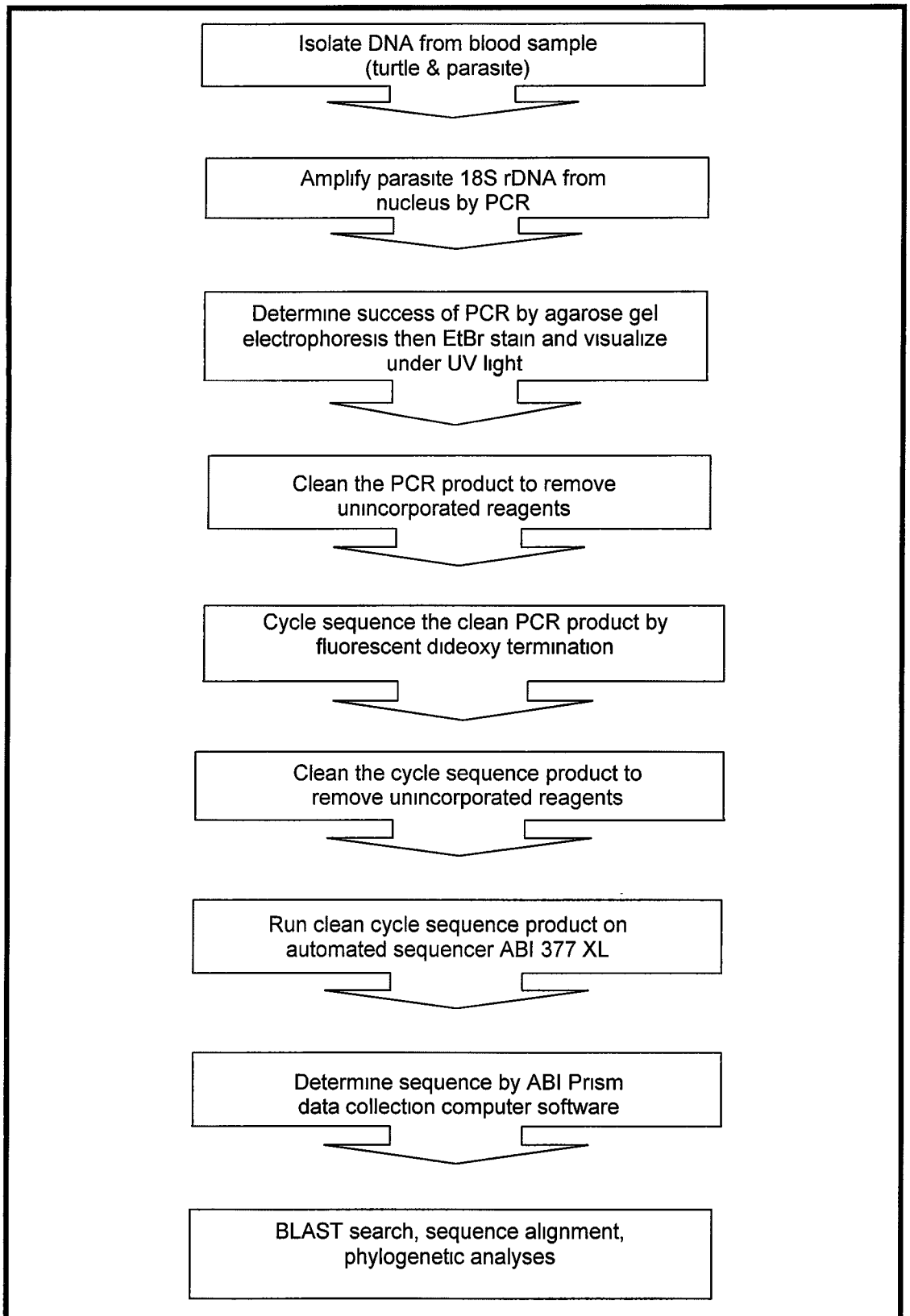


Figure 3 Overview of molecular methods for isolating *Haemogregarina* sp from various turtle host blood The parasite DNA was isolated, amplified by PCR, and sequenced

The *Haemogregarina*-specific primers developed were **18SF7** (forward) **5'-CAG TTG GGG GCA TTT GTA TTT AAC TGT CA- 3'** and **18SR6** (reverse) **5' - ATC TAT CCC CAT CAC GAT GCA YA- 3'.** These novel primers were used in the polymerase chain reaction (PCR) to amplify the 18S rRNA gene for apicomplexans. For chemical recipes see Appendix B. Amplifications were performed in 100 µl volumes containing 20.0 µl of Buffer A , 1.0 µl DMSO, 1.0 µl of 10 mM dNTPs , 1.0 µl forward primer 18SF7, 1.0 µl reverse primer 18SR6, 0.5 µl of 5µ/µl *Taq* polymerase, 1.0 µl template DNA, and 74.5 µl ddH_2O. Cycling parameters were 95 C for 30 seconds, 55 C annealing for 60 seconds, and 72 C extension for 60 seconds for 45 cycles using GeneAmp® PCR System 9700. Amplified products (10 µl) were separated on 1 % agarose gels, stained with 2% ethidium bromide solution, visualized using ultraviolet illumination and digitally photographed to record results. PCR products were purified to remove unincorporated salts, primers, buffers, and dNTPs with the Marligen® Rapid PCR Purification System (#11458-023) kit using the centrifugation protocol.

Clean PCR products were cycle sequenced with ddH_2O + template = 5.5 µl depending on the PCR product concentration compared to pGEM standard in agarose gel. For bidirectional cycle sequencing reactions, 0.5 µl sequencing primer, 3 µl Big Dye™ and varying amounts of template and ddH_2O were thermal cycled using GeneAmp® PCR System 9700 at 96 C for 30 seconds, 50 C for 60 seconds, and 60 C for 4 min for 25 cycles. Sequenced products were cleaned with Sephadex G-50 in CentriSep columns (Princeton Separations) by centrifugation to remove unincorporated ddNTPs and primers. Clean cycle sequence products were vacuum centrifuged to remove excess water.

Products were rehydrated in 1:5 ABI loading buffer to formamide solution. Products were analyzed on ABI PRISM™ 377 XL automated sequencer following included protocols. Resulting sequences were BLAST searched (www.ncbi.nlm.nih.gov) for species determination and aligned using Sequencher™ V4.1.2 (GeneCodes Corp.) with the final alignment adjusted by eye. See Appendix C for final alignment including *Haemogregarina* sequences resulting from this project, and see Appendix E for turtle alignment. All phylogenetic analyses were conducted using MacClade 3.05 (Maddison & Maddison, 1992) and PAUP* 4.0b10 (Swofford, 2002) and are discussed in Chapter 3.

RESULTS

Based on the morphology of the intraerythrocytic parasites, the diagnosis was determined to be either *Haemogregarina* sp. or *Hepatozoon* sp. since these were reported to parasitize chelonians. This judgment was based on previously published photographs and descriptions of the genera (Acholonu, 1974; Caskey, 1998; Desser, 1972; Hahn, 1909; Jakes et al., 2001; Marquardt, 1966; Paterson & Desser, 1976; Siddall & Desser, 1991; Telford et al., 2001; Wang & Hopkins, 1965). It is generally accepted that *Haemogregarina* occurs in turtles and is transmitted by a leech vector whereas *Hepatozoon* generally occurs in snakes and amphibians and is transmitted by mosquito or tick vector. Therefore without elucidation of the complete life cycle the diagnosis was determined as *Haemogregarina* sp. based on morphological characteristics and presumed leech vector. It is assumed that the leech is the intermediate host since the turtles are

aquatic and are exposed to leeches. It was not attempted to diagnose specific epithet of the haemogregarines based on morphological characteristics, however Chapter 3 discusses *Haemogregarina* nomenclature based on molecular characteristics.

Haemogregarina is distinguished primarily by its transmission by leech vectors. For many of the specimens for this study in wild populations, leeches were apparent on the shell and skin when turtles were bled. The leeches were identified as *Placobdella parasitica* and *Placobdella ornata* (Caskey, 1998; pers. comm. Christine Polito (SWTSU) to GLL, 2003; pers. comm. via Dr. Francis Rose to Liz Borda at American Museum of Natural History in New York, NY., 2002) The leeches were most often attached to posterior legs and were protected when the turtle legs were retracted beneath the shell.

The results for the blood smears and PCR based identification of *Haemogregarina* are shown in Table 5. All parasites were identified as *Haemogregarina* sp. for all infected turtles. For the three *Phrynops geoffroanus* individuals (MF5853, MF5854, and MF5855) the apicomplexan parasite *Haemoproteus* was identified (See Figure 15). The PCR method did not detect the *Haemoproteus* infection in these individuals. Many individuals exhibited an inclusion body or possible *Pirhemocyton* sp. virus (See Figure 13). Amplification attempts with primers from Smith et al. (1999) were unsuccessful with original parameters as well as modified cycling parameters.

Table 5 shows the comparison of results for detection of *Haemogregarina* sp. infection for microscopy and PCR techniques. All individuals were screened microscopically for parasites, however only approximately half of the individuals were screened by PCR for parasites.

Table 5. Comparison of results for detection of *Haemogregarina* sp. infection for microscopy and PCR techniques. All individuals were screened microscopically for parasites, however only approximately half of the individuals were screened by PCR for parasites.

Family	Included genera	# Indiv.	Microscopy	PCR
Chelidae (Austro-American side-neck turtles)	*Chelodina*	n=8	8/8 neg	4/4 neg
	Elseya	n=11	11/11 neg	6/6 neg
	Emydura	n=5	5/5 neg	3/3 neg
	Phrynops	n=6	6/6 neg	3/3 neg
	Platemys	n=2	2/2 neg	1/1 neg
Bataguridae (pond turtles)	*Callagur*	n=7	7/7 neg	3/3 neg
	Cyclemys	n=4	1/3 pos	1/2 pos
	Geoemyda	n=1	1/1 neg	1/1 neg
	Hardella	n=2	1/2 pos	1/2 pos
	Heosemys	n=20	19/20 pos	8/10 pos
	Hieremys	n=2	2/2 pos	1/1 pos
	Orlitia	n=10	8/10 pos	4/5 pos
	Rhinoclemmys	n=1	1/1 neg	1/1 neg
	Sacalia	n=2	no slides	2/2 neg
	Siebenrockiella	n=8	6/8 pos	2/4 pos
Emydinae (pond turtles)	*Chrysemys*	n=1	1/1 neg	1/1 neg
	Graptemys	n=5	3/5 pos	1/3 pos
	Malaclemys	n=4	4/4 neg	2/2 neg
	Pseudemys	n=48	33/48 pos	11/18 pos
	Trachemys	n=180	29/180 pos	8/19 pos
Kinosternidae (mud & musk turtles)	*Kinosternon*	n=3	2/3 pos	1/2 pos
	Staurotypus	n=7	6/6 neg	4/4 neg
	Sternotherus	n=3	3/3 pos	2/2 neg
Pelomedusidae (Afro-American side-necked turtles)	*Pelusios*	n=3	2/2 neg	2/2 neg
Trionychidae (softshell turtles)	*Apalone* (*Trionyx*)	n=1	1/1 neg	1/1 neg

Parasitemia in turtle sub-populations

All captive-raised individuals (n=160) were negative for *Haemogregarina* sp. infection. Both Guthrie Turtle Farms and Waterlife house multiple genera within large aquaria. Concordia Turtle Farm turtles included *Trachemys scripta elegans* and a single *Chrysemys picta* in which all individuals (n=141) were diagnosed as negative for *Haemogregarina* sp. parasitemia.

Parasitemia levels were determined by dividing the number of infected cells by the total number of cells counted. All wild caught individuals ranged in parasitemia levels from 0% to 3.42% with an average of 0.23% overall using microscopy identification. All captive individuals were negative for *Haemogregarina* infection, therefore all had 0% parasitemia levels.

Turtles collected from Spring Lake at Aquarena Springs included *Trachemys scripta elegans*, *Pseudemys texana* and *P. nelsoni*, and *Sternotherus odoratus*. The parasitemia for *Trachemys scripta elegans* (n=12) ranged from 0% to 1.67% (mean=0.53%). *Pseudemys texana* (n=31) ranged in parasitemia from 0% to 0.38% (mean=0.14%). *Pseudemys nelsoni* (n=2) ranged in parasitemia from 0.039% to 0.06% (mean=0.05%). The PCR detected parasites in all tested individuals with the exception of *Sternotherus odoratus* MF5497 (parasitemia level = 0.14%) and *S. odoratus* MF5502 (parasitemia level = 0.16%).

Individuals from Oasis Ranch in West Texas included *Pseudemys gorzugi*, *Trachemys scripta elegans*, and *Trionyx* (*Apalone*) *spinifera*. This location was the only wild population that was negative for *Haemogregarina* parasitemia. All individuals (n=17) from this locality were negative for parasitemia in both blood smears and PCR assay.

Turtles from the Capitol Aggregate locality in Marble Falls, Texas included 4 *Graptemys versa* and 1 *Trachemys scripta elegans*. Parasitemia ranged from 0 to 0.51% in *Graptemys* (mean=0.138%) and 0.09% for the single *Trachemys* specimen. PCR identification was accurate in identification of parasites for the two individuals assayed.

The single individual from Griffith League Ranch in Bastrop, Texas was a *Trachemys scripta elegans* in which microscopy determined parasitemia at 0.08%. The PCR assay was not performed on this individual.

The private ranch pond locality in Deanville, Texas contained exclusively (n=23) *Trachemys scripta elegans*. The parasitemia ranged from 0 to 1.88% and a mean of 0.28%. The PCR assay was accurate in amplification of haemogregarines with the exception to the individual MF5894 with a parasitemia level of 0.22%.

Wild-caught, captive-raised individuals were contained at Waterlife and Guthrie Turtle Farms. These turtles were classified into this category based on translocation within the past year from a wild population environment. Once turtles were in captivity

for a period of one year, then they were termed wild-caught, captive-raised (WCCR). The range of all WCCR parasitemia for 40 turtles (5 infected, 35 non-infected) was from 0 to 0.62% (mean =0.016%).

At the Guthrie Turtle Farm locale, the individuals meeting this criterion for WCCR included multiple genera. Included are *Siebenrockiella crassicollis* (n=2, parasitemia=0%) and *Cyclemys dentata* (n=4, parasitemia 0% to 0.003%, mean=0.0015%). *Sacalia bealeyi* (n=2) did not have blood smears, however PCR results confirmed that both individuals were negative for parasitemia. For *Rhinoclemmys punctularia* (n=1, parasitemia=0%), *Phrynops gibbus* (n=3, parasitemia=0%), and *Pelusios subniger* (n=3, parasitemia=0%). For *Platemys platycephala* (n=2, parasitemia=0%), and *Kinosternon sonorianse* (n=3, parasitemia = 0.03% to 0.62%, mean=0.325%). PCR diagnosed absence or presence of parasitemia in individuals where blood slides were not produced. The PCR assay was accurate in the amplification of the only turtle haemogregarine for this sub-population (*Kinosternon sonorianse* MF5513 for PCR assay, however MF5511 was amplified for sequence).

The turtle genera at Waterlife in Austin, Texas also included multiple genera classified as WCCR individuals. *Hardella thurjii* (n=2) in which one individual was positive (parasitemia=0.07%) and one individual was negative (0%) in which PCR analyses verified these results. *Phrynops geoffroanus* (n=3), *Elseya branderhorstii* (n=11), *Staurotypus triporcatus* (n=1), *Geoemyda nigricans* (n=1) *Chelodina mccordi*

(n=1), and *Emydura subglobosa* (n=1) were all negative for parasitemia and verified by PCR.

Digital images of *Haemogregarina* sp., *Haemoproteus* sp., and inclusion body or *Pirhemocyton* sp. infection were recorded. Figure 4 shows mature gamonts of *Haemogregarina clelandi* in *Emydura signata* taken from Jakes et al. (2001). The turtle host was collected from the freshwater Brisbane River in Queensland, Australia. This image shows the accepted morphology for *Haemogregarina* sp. in turtle erythrocytes. Figure 5 depicts *Haemogregarina sebai* from an African python taken from Hawkey & Dennett (1989). This image shows the accepted *Haemogregarina* morphology from a different source and reveals the identical morphology in an alternative host. Figure 6 shows the immature gamonts of *Haemogregarina* sp. from MF5810 *Pseudemys texana* from Aquarena Springs, San Marcos, Texas. Figure 7 shows multiple immature gamonts or merozoites of *Haemogregarina* sp. from MF5798 *Pseudemys texana* from Aquarena Springs, San Marcos, Texas.

Figure 8 depicts the immature gamonts of *Haemogregarina* sp. from *Pseudemys nelsoni* (MF5796) from Aquarena Springs, San Marcos, Texas. Figure 9 shows the mature gamonts of *Haemogregarina* from MF5814 *Heosemys grandis* rescued 3 months prior from Malaysia. This parasite form appears to have a parasitiphorous vacuole and is very large in size comparison to other haemogregarines. Figure 10 depicts the immature gamonts of *Haemogregarina* from MF5511 *Kinosternon sonorianse* obtained 5 years

prior from Malaysia. Figure 11 shows mature gamonts of *Haemogregarina* from MF5807 *Trachemys scripta elegans* Aquarena Springs, San Marcos, Texas.

Figure 12 depicts a published image of *Pirhemocyton* sp. from an Indian python from Hawkey & Dennett (1989) to use as morphologic comparison to this study. Figure 13 reveals a possible *Pirhemocyton* or inclusion bodies from MF5799 *Pseudemys nelsoni* from Aquarena Springs, San Marcos, Texas, however multiple genera exhibited this artifact or parasite. This individual was infected with *Haemogregarina* sp. (not shown). Figure 14 shows the accepted morphology of *Haemoproteus chelodinae* in *Elseya latisternum* from Jakes et al. (2001) to use as morphologic comparison in this study. Figure 15 shows *Haemoproteus* sp. infection in *Phyrnops geoffroanus* (MF5853) from Waterlife captive facility. It appeared as a white foamy substance that occasionally displaced the nucleus.

Table 6 shows the sequences at corresponding primer sites previously reported for successfully isolating haemogregarines from vertebrate hosts. See Wozniak et al. (1994); Mathew et al. (2000); Smith et al. (1999) for primer reference and sequence. The primers 18SF7 and 18SR6 were the primers used in the current study to isolate *Haemogregarina* sp. from turtle blood. Vertebrates were included in the alignment to develop primers specific for the parasite DNA and excluding the host DNA. In the case of an ambiguous vertebrate base (N) for turtle sequence, an alternative vertebrate (*Alligator*) base was substituted for this table. The Wozniak et al. (1994) primers reveal 100% identity to

Figure 4. Mature gamonts of *Haemogregarina clelandi* in *Emydura signata* from Jakes et al. (2001). Bar = 8 μm. The turtle host was collected from the freshwater Brisbane River in Queensland, Australia. RBC size appx. 20 μm x 12 μm.

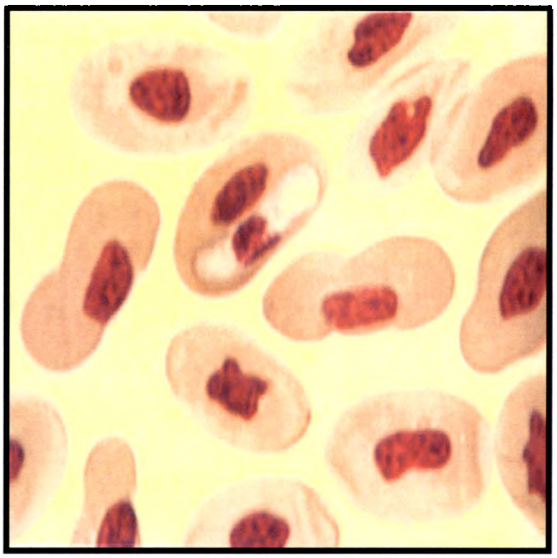


Figure 5. *Haemogregarina sebai* from an African python from Hawkey & Dennett, (1989). The dark-staining nucleus of the parasite is evident in contrast to the host nucleus. RBC size appx. 20 μm x 12 μm.

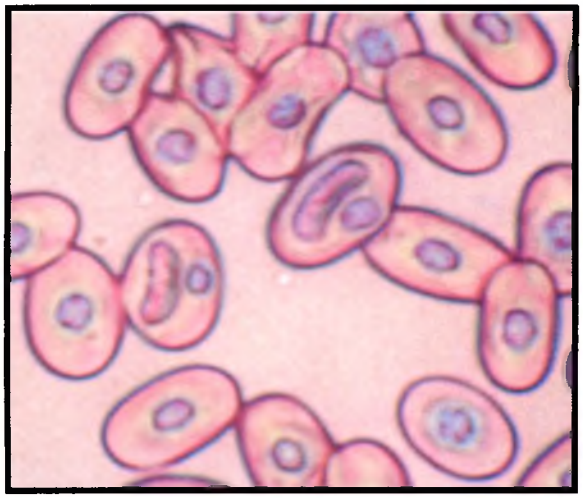


Figure 6. Immature gamonts of *Haemogregarina* sp. from MF5810 *Pseudemys texana* from Aquarena Springs, San Marcos, Texas. Note the curved parasites that have displaced the host nucleus. RBC size appx. 20 μm x 12 μm.

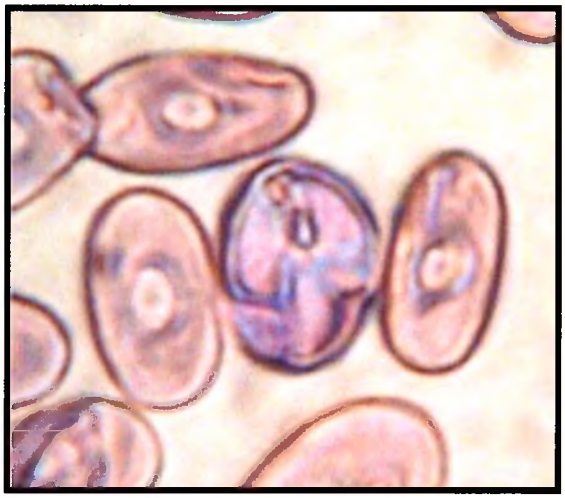


Figure 7. Multiple immature gamonts or merozoites of *Haemogregarina* sp. from MF5798 *Pseudemys texana* from Aquarena Springs, San Marcos, Texas. Note the three parasites within a single erythrocyte that have displaced the host nucleus. RBC size appx. 20 μm x 12 μm.

Figure 8. Immature gamonts of *Haemogregarina* sp. from MF5796 *Pseudemys nelsoni* from Aquarena Springs, San Marcos, Texas. Note the two parasites occupying a single erythrocyte that have displaced the host nucleus. RBC size appx. 20 µm x 12 µm.

Figure 9. *Haemogregarina* from MF5814 *Heosemys grandis* obtained 3 months prior from Malaysia. Note the parasitiphorous vacuole surrounding the very large parasite. From this perspective, it is not clear if the parasite is intra- or extra-erythrocytic. RBC size appx. 20 µm x 12 µm.

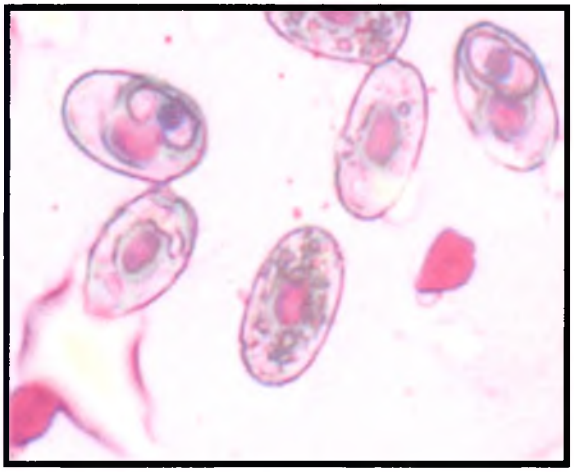


Figure 10. Immature gamonts of *Haemogregarina* from MF5511 *Kinosternon sonorianse* seized 5 years prior from Malaysia. Note the two parasites with the dark staining nucleus that has slightly displaced the nucleus. RBC size appx. 20 µm x 12 µm.

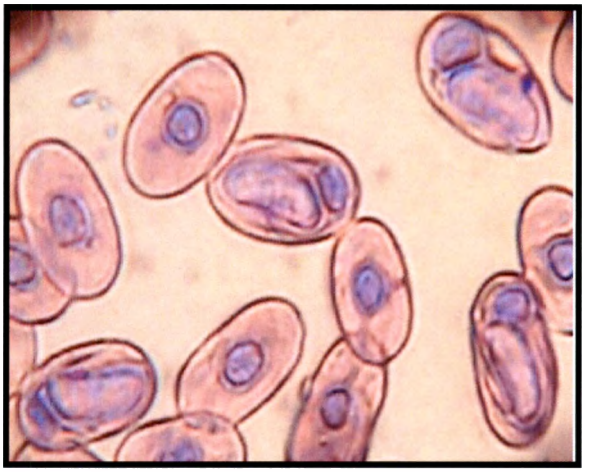


Figure 11. Mature gamonts of *Haemogregarina* from MF5807 *Trachemys scripta elegans* Aquarena Springs, San Marcos, Texas. Note the four parasites in this one view as well as the host nucleus displacement. RBC size appx. 20 µm x 12 µm.

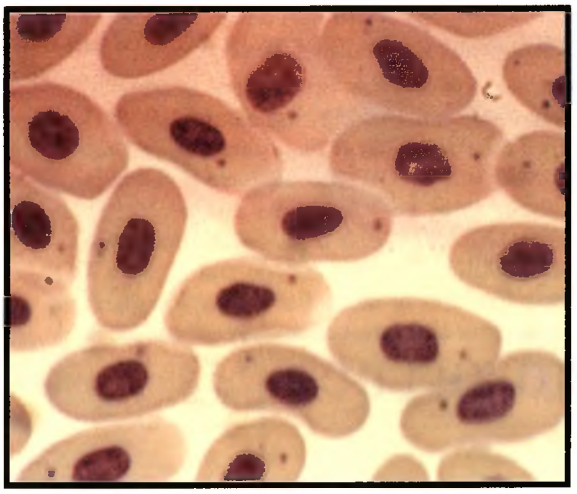


Figure 12. *Pirhemocyton* sp. from an Indian python from Hawkey & Dennett (1989). *Pirhemocyton* may be a virus that must be examined with electron microscopy, or may be an inclusion body within the cell. RBC size appx. 20 µm x 12 µm.

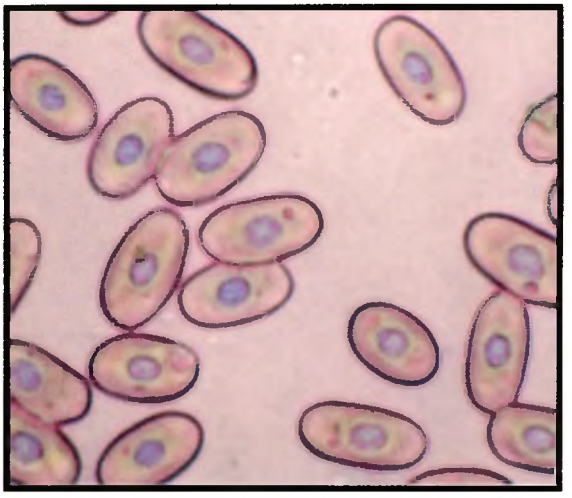


Figure 13. Possible *Pirhemocyton* or inclusion bodies from MF5799 *Pseudemys nelsoni* from Aquarena Springs, San Marcos, Texas. This individual was infected with *Haemogregarina* sp. (not shown). RBC size appx. 20 µm x 12 µm.

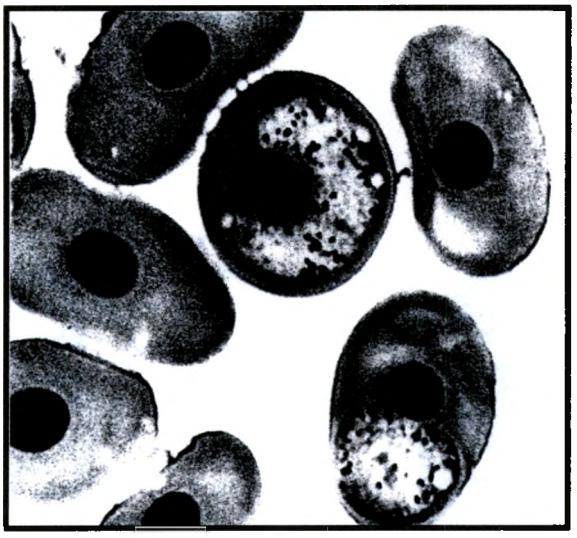


Figure 14. *Haemoproteus chelodinae* in *Elseya latisternum* from Jakes et al. (2001). *Haemoproteus* is an Apicomplexan parasite. RBC size appx. 20 µm x 12 µm.

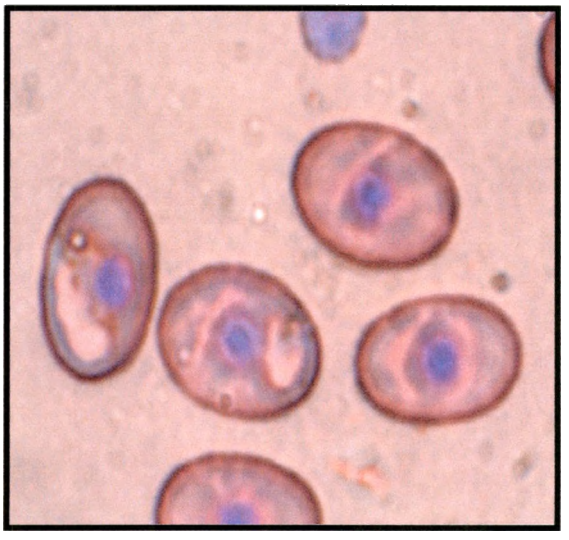


Figure 15. *Haemoproteus* in *Phyrnops geoffroanus* (MF5853) from Waterlife captive facility. It appeared as a white foamy substance that occasionally displaced the nucleus. RBC size appx. 20 µm x 12 µm.

both turtle and parasite regions. The Mathew et al. (2000) 4558 primer contains an 11 base region with 100% identity then the following 13 base region with 31% identity with turtle sequence. The Mathew et al. (2000) 2733 primer is 100% identical with turtle, and 4374 primer is 94% identical to the host and differs by a single base. The Mathew et al. (2000) 4559 primer is 65% identical overall, and 100% identical for the last 10 bases (at the 3' end) of the primer sequence region.

For the Smith et al. (1999) forward primer, the ITS-1 region was used (which was not included in the 18S alignment) therefore see Appendix D for ITS-1 region alignment; *Xenopus* (Gen Bank #X02995) was the only vertebrate sequence available for alignment purposes for this region. Both ITS-7 and ITS-8 primers (Smith et al., 1999) show 98% identity to both *Xenopus* and *Hepatozoon* sequence. The primers designed for the current analyses included 18SF7 and 18SR6, which overall show 63% and 27% identity respectively to the turtle sequence.

Figures 16 through 20 show the results of PCR amplification of *Haemogregarina* sp. from turtle blood. On the 1% agarose gel, a primer band indicates positive result, whereas lack of a band indicates a negative result. Sample numbers are given in each lane and turtle genera are abbreviated in captions. For some lanes with very light bands, the actual gel was observed to determine accurately the positive or negative result. The following genera were observed as positive for parasitemia in the blood smears, however

Table 6 Sequences at corresponding primer sites previously reported for successfully isolating haemogregarines from vertebrate hosts See Wozniak et al , 1994, Mathew et al , 2000, Smith et al , 1999 The primers 18SF7 and 18SR6 were the primers used for this study to isolate *Haemogregarina* sp Vertebrates were included in the alignment to develop primers specific for the parasite and excluding the host In the case of an ambiguous vertebrate base (N) for turtle sequence, an alternative vertebrate (*Alligator*) base was substituted for this table For Smith forward primer, the ITS-1 region was used (which was not included in the 18S alignment) therefore see Appendix D for ITS-1 region alignment, *Xenopus* (Gen Bank #X02995) was the only vertebrate sequence available for alignment purposes for this region Reverse primers have been reversed and complemented to show alignment with sequence

Wozaniak et al., 1994:

Turtle sequence	**5'- GAG TAA ATT AGA GTG TTC CAA GCA -3'**
***Hepatozoon* sequence**	**GAG AAA ATT AGA GTG TTT CAA GCA**
18AP853.F forward	**GAG TAA ATT AGA GTG TTC CAA GCA**
Turtle sequence	**5'- GAT TTG TCT GGT TAA TTC CG -3'**
***Hepatozoon* sequence**	**GAT TTG TCT GGT TAA TTC CG**
18AP1488.R reverse	**GAT TTG TCT GGT TAA TTC CG**

Mathew et al., 2000:

Turtle sequence	**5'- GCT AAT ACA TGC CGA CGA GCG CTG -3'**
***Hepatozoon* sequence**	**GCT AAT ACA TGA GCA AAA TCT CAA**
4558 external forward	**GCT AAT ACA TGA GCA AAA TCT CAA**
Turtle sequence	**5'- CGG AAT TAA CCA GAC AAA T -3'**
***Hepatozoon* sequence**	**CGG AAT TAA CCA GAC AAA T**
2733 external reverse	**CGG AAT TAA CCA GAC AAA T**
Turtle sequence	**5'- ATC CAA GGA AGG CAG C -3'**
***Hepatozoon* sequence**	**ATC TAA GGA AGG CAG C**
4374 internal forward	**ATC TAA GGA AGG CAG C**
Turtle sequence	**5'- CCG ACC CGG GGA GGT AGT GA -3'**
***Hepatozoon* sequence**	**CAG CAT AAA AGA GGT AGT GA**
4559 internal reverse	**CAG CAT AAG AGA GGT AGT GA**

Smith et al., 1999:

***Xenopus* sequence**	**5'- CCG TAG GTG AAC CTG CGG AAG -3'**
***Hepatozoon* sequence**	**CGG TAG GTG AAC CTG CGG AAG**
ITS-7 forward	**CGG TAG GTG AAC CTG CGG AAG**
***Xenopus* sequence**	**5'- GCA TCG ATG AAG GAC GCA GC -3'**
***Hepatozoon* sequence**	**GCA TCG ATG AAG GAC GCA GC**
ITS-8 reverse	**GCA TCG ATG AAG GAC GCA GC**

Current analyses:

Turtle sequence	**5'- CGG CCG GGG GCA TTC GTA TTG TGC CGC TA -3'**
***Hepatozoon* sequence**	**CAG TTG GGG GCA TTT GTA TTT AAC TGT CA**
18SF7 forward	**CAG TTG GGG GCA TTT GTA TTT AAC TGT CA**
Turtle sequence	**5'- ACC CCA TTC GTG ATG GGN ATC GGG -3'**
***Hepatozoon* sequence**	**TGT GCA T :C GTG ATA GGA ATA GAT**
18SR6 reverse	**TRT GCA TCG TGA TGG GGG ATA GAT**

resulted as negative for parasitemia in the PCR assay:

- MF#5826 *Hieremys annandelli* (parasitemia=0.61%)
- MF#5513 *Kinosternon sonorianse* (parasitemia=0.03%)
- MF#5460 *Pseudemys texana* (parasitemia=0.16%)
- MF#5463 *Pseudemys texana* (parasitemia=0.01%)
- MF#5464 *Pseudemys texana* (parasitemia=0.25%)
- MF#5497 *Sternotherus odoratus* (parasitemia= 0.14%)

There were not any blood smears deemed as negative that resulted in a positive PCR result.

The statistical significance of infections between populations were evaluated by pairwise comparisons between captive, wild, and WCCR (wild caught, captive raised) populations. For captive to wild populations, WCCR to wild populations, and captive to WCCR populations there was no difference between the populations based on the confidence interval values. For the microscopy method to PCR method within Chelidae and separately within Emydinae, there was no difference between the populations based on the confidence interval values. However, for the microscopy method to PCR method within Bataguridae and separately within Kinosternidae there is a difference between populations based on the confidence interval values. Table 7 shows the confidence interval values for pairwise comparisons.

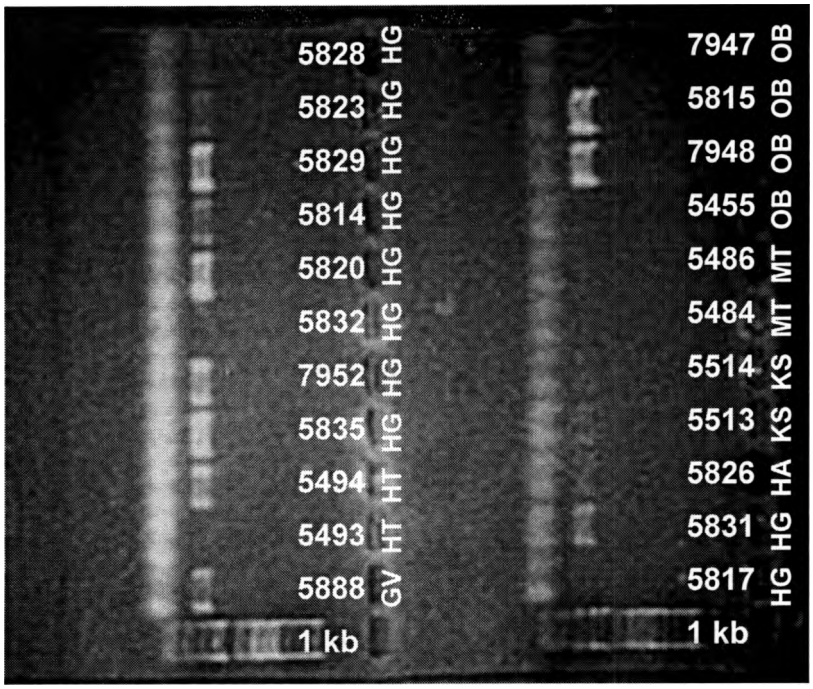


Figure 17. Results of PCR amplification of *Haemogregarina* sp. from turtle blood. Primer band indicates positive result, whereas lack of a band indicates a negative result. Sample numbers are given in each lane. GV=*Graptemys versa*, HT=*Hardella thurjii*, HG=*Heosemys grandis*, HA=*Hieremys annandalii*, KS=*Kinosternon sonoriense*, MT=*Malaclemys terrapin*, OB=*Orlitia borneensis*. The migration of the molecules on the gel runs from the well (labeled with sample number) towards the top of the gel.

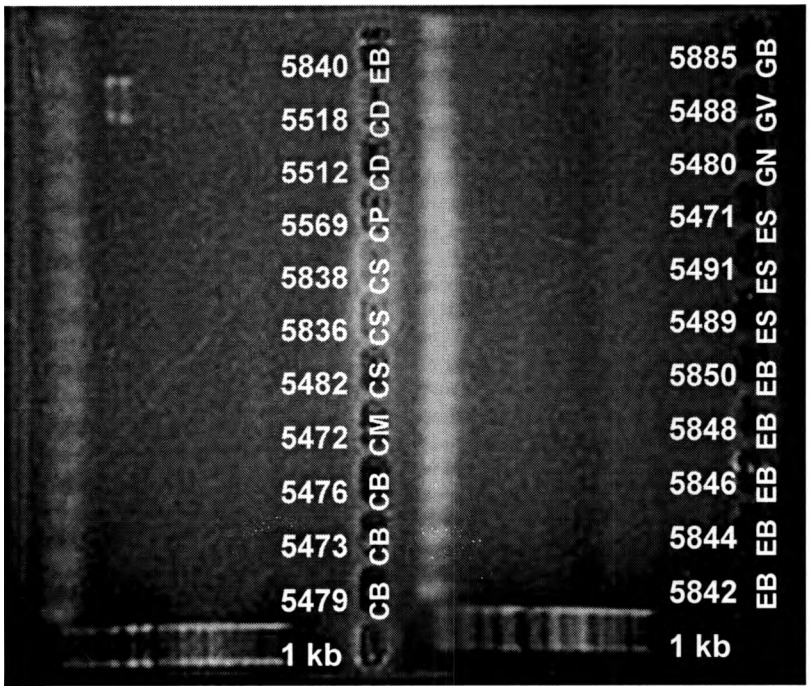


Figure 16. Results of PCR amplification of *Haemogregarina* sp. from turtle blood. Primer band indicates positive result, whereas lack of a band indicates a negative result. Sample numbers are given in each lane. CB=*Callagur borneoensis*, CM=*Chelodina mccordi*, CS=*Chelodina siebenrocki*, CP=*Chrysemys picta*, CD=*Cyclemys dentata*, EB= *Elseya branderhorstii*, ES=*Emydura subglobosa*, GN=*Geoemyda nigrican*, GV=*Graptemys versa*, GB=*Graptemys barberi*. The migration of the molecules on the gel runs from the well (labeled with sample number)

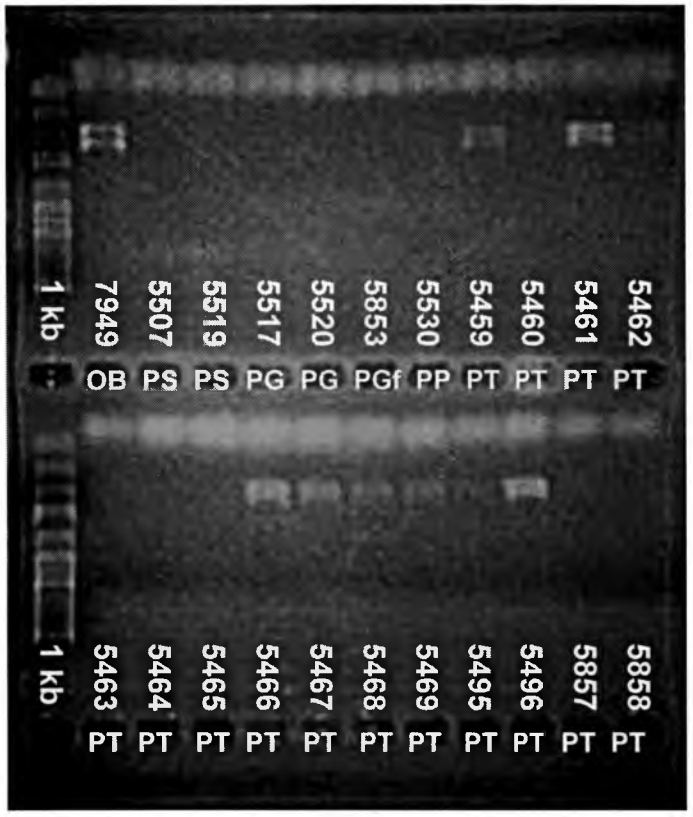


Figure 18. Results of PCR amplification of *Haemogregarina* sp. from turtle blood. Primer band indicates positive result, whereas lack of a band indicates a negative result. Sample numbers are given in each lane. OB= *Orlitia borneensis*, PS=*Pelusios subniger*, PG=*Phrynops gibbus*, PGf=*Phrynops geoffroanus*, PP=*Platemys platycephala*, PT=*Pseudemys texana*. The migration of the molecules on the gel runs from the well (labeled with sample number) towards the top of the gel.

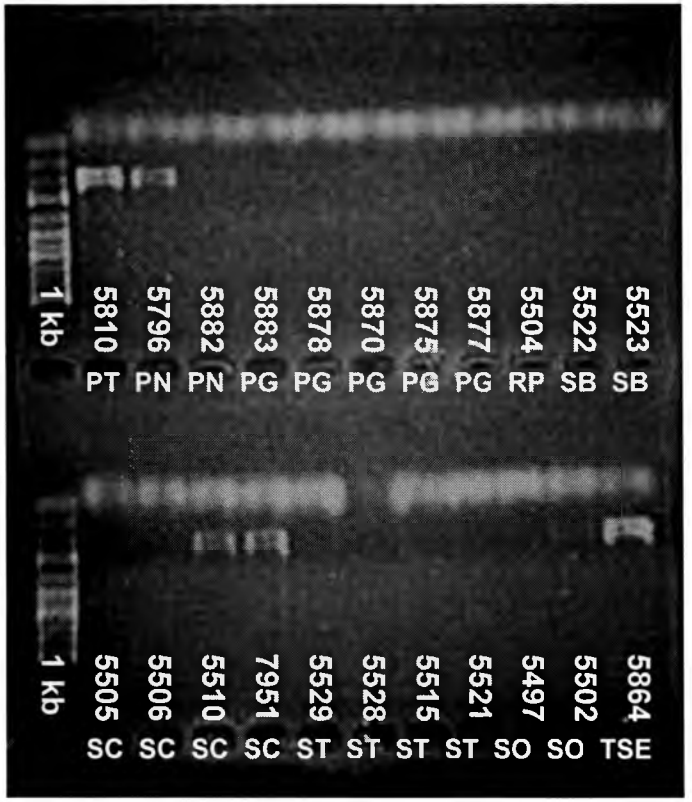


Figure 19. Results of PCR amplification of *Haemogregarina* sp. from turtle blood. Primer band indicates positive result, whereas lack of a band indicates a negative result. Sample numbers are given in each lane. PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, PG=*Phrynops gibbus*, RP=*Rhinoclemys punctilaria*, SB=*Sacalia bealeyi*, SC=*Siebenrockiella crassicollis*, ST=*Staurotypus triporcatus*, SO= *Sternotherus odoratus*, TSE=*Trachemys scripta elegans*. The migration of the molecules on the gel runs from the well (labeled with sample number) towards the top of the gel.

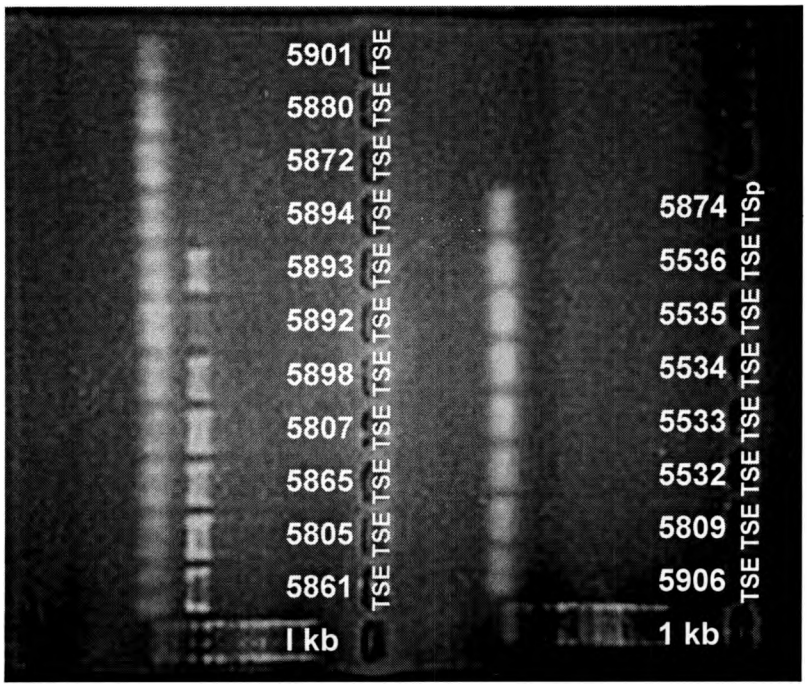


Figure 20. Results of PCR amplification of *Haemogregarina* sp. from turtle blood. Primer band indicates positive result, whereas lack of a band indicates a negative result. Sample numbers are given in each lane. TSE=*Trachemys scripta elegans*, TSp=*Trionyx* (*Apalone*) *spinifera*. The migration of the molecules on the gel runs from the well (labeled with sample number) towards the top of the gel.

Table 7. Pairwise comparisons of confidence intervals among for turtle sub-populations. p_1=proportion of infected individuals for group 1, p_2= proportion of infected individuals for group 2, n_1=total number of individuals for group 1, n_2=total number of individuals for group 2. In a pairwise comparison, if the upper bound and lower bound include 0, there is not a difference between the two groups. If the upper bound and lower bound do not include 0, then there is a difference between the two groups.

	p_1	n_1	p_2	n_2	formula	upper bound	lower bound	difference between groups
Captives to Wild	0	160	0.76136	228	0.0553	0.8167	-0.7060	no
WCCR to Wild	0.125	40	0.76136	228	0.1165	1.0028	-0.5199	no
Captives to WCCR	0	160	0.125	40	0.1025	0.2275	-0.0225	no
Microscopy vs. PCR for Chelidae	0	32	0	17	0	0	0	no
Microscopy vs. PCR for Bataguridae	0.69811	53	0.54839	31	0.2144	1.4609	0.3641	yes
Microscopy vs. PCR for Emydinae	0.27311	238	0.46511	43	0.1595	0.8977	-0.0325	no
Microscopy vs. PCR for Kinosternidae	0.41667	12	0.125	8	0.3610	0.9027	0.6527	yes

DISCUSSION

All individuals (100% of 160 turtles) from captive populations were negative for parasitemia. For these captive populations, parasitological control of leeches had historically been performed through the addition of copper sulfate which appears to be correlated with absence of parasitemia. Wild populations, however, do not have any method of leech vector control and the different localities show varying degrees of haemogregarine parasitemia. Turtles that were imported from Asia (wild populations) harbored leeches that potentially could have infected the captive populations that were not exposed to the vector. The WCCR individuals that were positive for *Haemogregarina* sp. probably had an existing infection prior to translocation and were not infected upon arrival to the captive ponds. The two leeches identified from Spring Lake at Aquarena Springs were *Placobdella ornata* and *P. parasitica*. It is not known, however, if there is any host specificity of the haemogregarines to the leeches.

Method comparison

The microscopy method compared to the PCR method revealed that 6 out of 107 (5.6%) individuals were positive with microscopy but failed to prove positive in the PCR assay. For low-level parasitemia, however, it is essential to use "enough" blood for the initial DNA extraction to acquire the haemogregarine parasite. In an estimation to compare the amounts of blood per assay, it was calculated approximately that 10µl of blood was used for each blood smear whereas approximately 5µl of only blood

(excluding the blood storage buffer) was used for the DNA extraction. Perhaps an increase in the amount of the blood-blood storage buffer solution would increase the accuracy of the PCR assay. It should be noted, however, that negative blood smears and negative PCR assessments do not necessarily eliminate the possibility of an infection that is too low to detect. It is suggested that both blood smears as well as PCR is used for future *Haemogregarina* studies.

It is interesting to note the molecular problems encountered previously for amplification of *Haemogregarina* (or *Hepatozoon*) genera. Table 6 shows that a majority of the alleged success for haemogregarines may in fact be amplification of the host. Given the fact that reptilian erythrocytes are nucleated, there is a significant amount of DNA that is isolated with the parasite DNA in extraction from whole blood. In the estimate of a low infection of 0.20%, the amount of host DNA would be 500 times more than the amount of parasite DNA. An evaluation of the primer sites (see Table 6) used in Wozniak et al. (1994) reveal that the primers are 100% conservative for the host and the parasite. Given the vast amount of host DNA, it is probable that Wozniak et al. (1994) amplified the host. Mathew et al. (2000) used internal and external primers to gain parasite specific regions (see Table 6). The Mathew et al. (2000) 4558 external appears to exclude the host, however the 2733 external primer is 100% identical to both host and parasite. In an evaluation of Mathew et al. (2000) internal primers, the 4374 internal is almost identical to both host and parasite gene regions. The Mathew et al. (2000) 4559 internal has differentiation between host and parasite, which occurs at the 5' region. However, the binding specificity is much more important at the 3' region for primer

sequence. For the Mathew et al. (2000) study, it is uncertain whether the parasite was indeed isolated. In a similar study, Smith et al. (1999) evaluated molecular identification of haemogregarines. An evaluation of Table 6 with the only vertebrate ITS region available reveals 100% identity to both host and parasite genes. This, as well as Wozniak et al. (1994), prove that amplification of only the parasite and not the host is mathematically unfeasible. An examination of the primers used for the current study (See Table 6) show variability at the 3' sequence region that is necessary for amplification of *Haemogregarina*.

Haemogregarina infections that had not previously been reported for the following turtle taxa (most of which are translocated turtles) are:

- *Cyclemys dentata*
- *Graptemys versa*
- *Hardella thurjii*
- *Heosemys grandis*
- *Hieremys annandalii*
- *Kinosternon sonoriense*
- *Orlitia borneensis*
- *Siebenrockiella crassicollis*

Prevention of transmission

Conservation strategies involving captive husbandry may potentially create infections in unnatural hosts and thus decrease the viability of these captive populations. The recently-obtained turtles from Asia (within the past three months) all were positive for *Haemogregarina* infections. This parasite could potentially induce a pathogenic response in the captive-maintained turtles if the parasite differs significantly from the haemogregarines in which these turtles were previously exposed. Haemogregarines are speculated to not be pathogenic in their natural hosts, however they may be pathogenic in unnatural hosts. Until further transmission studies and host specificity are determined, it is safe to consider turtles in a new environment to be unnatural hosts and thus the parasites as potential pathogens. Captive management is essential for the survival of these endangered turtles, however strict disease control methods are vital.

Most animals are translocated for three reasons: reintroduction of native species that have become extinct in a locality, increasing low populations of individuals, and rehabilitating illegally captured and confiscated organisms (Woodford, 2000). In order to effectively translocate endangered turtles to promote biodiversity through conservation, an accurate and successful strategy to promote the welfare of the turtles is essential. One of the many factors that must be evaluated is the presence of disease that may infect the resident population. Researchers consider the translocated organism as more than a single individual but "as a 'package' containing an assortment of potentially dangerous viruses, bacteria, protozoa, helminths and arthropods, any of which may become

pathogenic in a new situation, involving stressed individuals in a changed environment" (Griffith et al., 1989).

Woodford (2000) outlines protocols for effective quarantine and health evaluations preceding the translocation of animals. This includes a full health evaluation, a quarantine interlude, and pre-release treatment including vaccinations when available. The introduction of disease during translocation has the capacity to reverse the possible benefits of the captive breeding programs by causing death, immunocompromised individuals at risk to other diseases, and decrease in reproduction (Cunningham, 1996). An introduction of new parasites (to potentially new hosts) may also disturb the host-parasite relationship (Cunningham, 1996) thereby affecting the ecology of the new environment. Control of the leech vector does appear to control the spread of *Haemogregarina* infection and persistence.

For *Haemogregarina* sp. infections involving seized turtles, the pathogenicity of this parasite should be determined for the pathological effects in natural and unnatural hosts. Other factors that need to be considered are the length of infection for a particular host, chemotherapy treatment, the level of infection that can be tolerated, and in-depth transmission studies involving associated vectors. This study is a preliminary perspective considering the species infected and parasite detection for further analyses.

CHAPTER III

PHYLOGENETIC ANALYSES

OF *HAEMOGREGARINA*

Comparison among multiple species reveal information on the adaptive processes and evolutionary histories, thus revealing the chronology of evolutionary events. Phylogenetic analyses map these evolutionary events based on synapomorphic characters. Preceding to the application of molecular phylogenetics, apicomplexans were identified and classified solely on their morphological characters and life history. This included size and shape of various life cycle stages and the location of infection. Morphological characteristics of apicomplexans that were used for systematic classification and phylogenetic analyses included ultrastructural characteristics, shape and size of various life stages, location of gamonts in host cell, and type of definitive host (Smith et al., 2000; Siddall & Desser, 1991). Morphology, vertebrate host, invertebrate host, ecological and biogeographical conditions were utilized to produce phylogenies. This proves to be problematic, however, due to ambiguous terminology, functional speculation, the determination and eventual assignment of primitive or derived characters, and in establishing homology. Today both morphological and molecular data are used in identification, systematic classification and phylogeny reconstructions.

In phylogenetic reconstruction, there are five conditions in which the use of only morphological data are insufficient for distinguishing species boundaries (Hillis et al., 1996):

1) Species may be sympatric (overlapping) or parapatric (adjacent to), but differ significantly in morphology and remain unnoticed as a species unit (Donnellan and Aplin, 1989).

2) Allopatric (geographically isolated) populations may also remain unnoticed as a species unit when considering the Biological Species Concept.

3) Parametric populations could represent morphologically distinct isolates however crossbreeding occurs (Jackman & Wake, 1994).

4) Populations with morphologically distinguishable isolates merely represent variation in the single interbreeding population (Hillis et al., 1991).

5) Morphologically similar groups represent individual speciation events and show convergence in morphologic characters.

Phylogenetic reconstruction of apicomplexans based solely on morphological characters leads to difficulties in species determination based on highly conserved ultrastructure and life histories, an *a priori* concept of primitive or derived characters, ambiguous descriptive terms, determination of homology, and multiple host-switching

events. Molecular characters used for phylogenetic reconstruction, however, provide explicit and definable characters (only 4 nucleotides) that may be used to determine homology. Both morphological and molecular data are limited with respect to convergence, however, as the erroneous homology is due to a similar evolutionary change.

For identification and phylogenetic analyses in the Apicomplexa, ribosomal DNA genes have been used including 18S, 5.8S, and ITS regions (Cai et. al, 1992; Goggin, 1994; Kim et al., 1998; Mathew et al, 2000; Moon-van der Staay, 2001; Perkins & Keller, 2001; Smith et al., 1999; and Wozniak & McLaughlin, 1993). The small subunit ribosomal DNA from the apicoplast genome has also proven to resolve taxonomic differences and phylogenies (Obornik et al., 2002). These genes can be used to determine morphologically indistinguishable but otherwise distinct species (Hillis & Dixon, 1991). Figure 21 shows a diagram of the ribosomal DNA gene family in eukaryotes (Hillis & Davis, 1986).

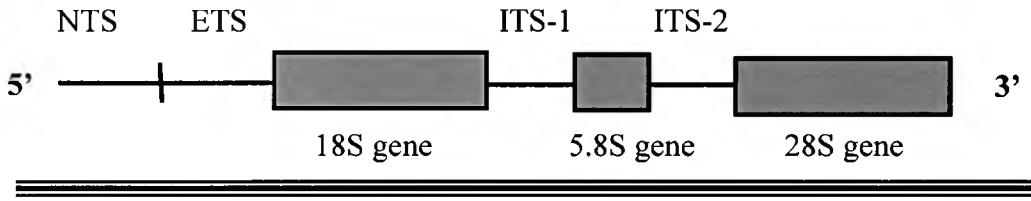


Figure 21. Diagram of the ribosomal DNA gene family in eukaryotes (Hillis & Davis, 1986). The shaded blocks show the 18S, 5.8S, and 28S genes coding regions. The NTS is the nontranscribed spacer, the ETS is the external transcribed spacer, and the ITS regions are the internal transcribed spacer regions. In eukaryotes, the 18S = 1800 nt, 5.8S = 160 nt, and 28S = over 4000 nt.

The small subunit of ribosomal DNA is often employed to determine phylogenetic relationships of eukaryotes. This molecule's attributes include its conservation due to functional constraints, variable mutation rates in different substructure positions, and its ubiquity among all taxa (Ouvrard et al., 2000). The 18S subunit is an established region containing both low substitution rates as well as regions of high substitution rates. This allows for phylogenetic resolution of organisms of deep divergence (between classes) as well as recent divergence (between species). The approximate length in eukaryotes for the ribosomal genes are 18S = 1800 nt, 5.8S = 160 nt, and 28S = over 4000 nt. (Hillis & Dixon, 1991). There are two internal transcribed spacers: one between 18S and 5.8S and the other between 5.8S and 28S. The transcribed spacers contain signals for processing the rRNA transcript (Hillis & Dixon, 1991).

There have been numerous attempts at phylogenetic reconstruction based on apicomplexan morphology and life history (Mathew et al., 2000; Siddall, 1995; Barta, 1989), however morphologic comparisons were not covered in the scope of this project. There were far fewer efforts at phylogenetic reconstruction based on molecular characters within the Apicomplexa. Most phylogenies were limited to an inadequate number of taxa under investigation for a phylum-level study. Table 6 features a list of genera evaluated in previously published in molecular phylogenetic analyses within Apicomplexa The analyses in this study are the most taxon inclusive evaluating 18S nrDNA for Apicomplexa. Leander et al. (2003) used 18S and beta-tubulin and included Apicomplexans in addition to Ciliates, Perkinsids, and Dinoflagellates. Mathew et al. (2000) utilized ITS regions and 18S rRNA with fewer taxa and Morrison & Ellis (1997)

also evaluated 18S rDNA. Obornik et al. (2002) examined apicoplast (degenerate plastid genome which lacks genes for photosynthesis) rDNA, and Perkins & Keller (2001) used the mitochondrial gene region Cytochrome b.

The only genera of Apicomplexa accessible through GenBank (see Table 8) that were not included in sequence alignment or phylogenetic analyses for this study include *Ascogregarina*, *Besnoitia*, *Choleoeimeria*, *Frenkelia*, *Haemoproteus*, *Hepatocystis*, *Lecudina*, *Leucocytozoon*, and *Goussia*. This was due to their sequence from a gene that did not include 18S or the inability to fit their sequence into the final alignment due to extreme sequence divergence. GenBank does not currently have *Haemogregrina* sequence, therefore novel primers were developed and sequence successfully generated for phylogenetic analyses. See chapter 2 for molecular methods and sequence generation.

Morphologic analyses

The numerous attempts at phylogenetic reconstruction for apicomplexans morphology were historically based on morphology. Although morphological characters were not examined in the current study, it is useful to examine morphologic synapomorphies as additional support of phylogenies. Figure 22 depicts the consensus tree of the 2 most parsimonious trees resulting from a phylogenetic analysis of 26 morphologic characters (Smith et al., 2000). This tree uses coccidia *Klossia* and *Adelina* as outgroups and shows most isolates of *Hepatozoon* as a monophyletic group. Sister

Table 8. List of genera evaluated in molecular phylogenetic analyses within Apicomplexa. The current analyses are the present study evaluated 18S rDNA. Leander et al. (2003) used 18S and beta-tubulin and included Apicomplexans in addition to Ciliates, Perkinsids, and Dinoflagellates. Morrison & Ellis, (1997) evaluated 18S rDNA. Mathew et al. (2000) also utilized 18S rRNA. Obornik et al. (2002) evaluated apicoplast rDNA for Apicomplexans and algal plastid lineages. Perkins & Keller (2001) examined Cytochrome b.

	Current analyses	Leander et al., 2003	Morrison & Ellis, 1997	Mathew et al., 2000	Obornik et al., 2002	Perkins & Keller, 2001
Babesia	✓	✓	✓	✓	✓	✓
Caryospora	✓					
Colpodella	✓	✓				
Cryptosporidium	✓	✓	✓	✓		✓
Cyclospora	✓			✓		
Cytauxzooon	✓	✓	✓	✓		
Eimeria	✓	✓	✓	✓	✓	✓
Gregarina	✓	✓		✓		
Haemogregarina	✓					
Hammondia	✓					
Hepatozoon	✓			✓	✓	✓
Hyaloklossia	✓				✓	
Isospora	✓			✓		
Lankesterella	✓					✓
Lecudina		✓				
Leidyana	✓	✓				
Monocystis	✓	✓				
Neospora	✓	✓	✓	✓	✓	
Ophriocystis	✓	✓				
Plasmodium	✓		✓	✓	✓	✓
Pseudomonocystis	✓					
Sarcocystis	✓	✓	✓	✓	✓	✓
Theileria	✓	✓	✓	✓		✓
Toxoplasma	✓	✓	✓	✓	✓	✓
Total	**23**	**14**	**9**	**13**	**8**	**9**

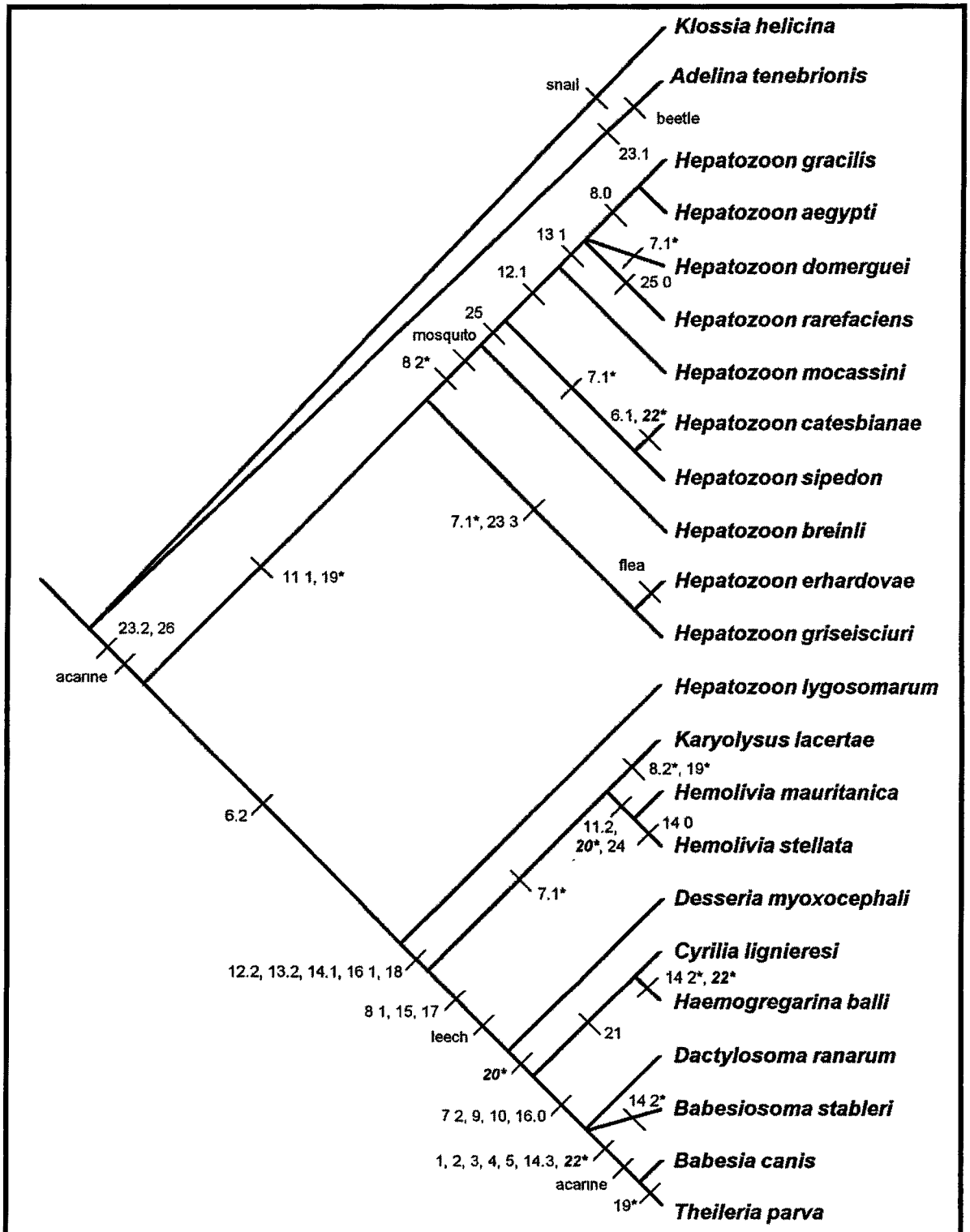


Figure 22. Consensus tree of the 2 most parsimonious trees resulting from a phylogenetic analysis of 26 [morphologic] characters Length = 50 steps, CI=0.72, RI=0.89. Transformation numbers correspond to characters listed in Table 1 [of Smith et al. 2000], with hypothesized homoplasy events indicated by asterisks. The type of invertebrate host (character 27) utilized by each species is mapped onto the trees constructed by analysis of the first 26 characters. Entire diagram and legend from Smith et al. (2000) as comparison of previously accepted morphologic phylogenetic tree.

group to the *Hepatozoon* includes a *Karyolysus-Hemolovia* clade, sister to *Desseria*, sister to *Cyrilia-Haemogregarina* clade.

The currently accepted systematics for Apicomplexa used in this study are outlined in Table 9. Apicomplexans that did not have sequence in GenBank, with the exception of *Haemogregarina,* could not be added to the molecular phylum-level study.

Molecular analyses

Previous phylogenetic hypotheses of Apicomplexa based on DNA sequence variation of the 18S rRNA gene are depicted in Figure 22 (Mathew et al., 2000). This cladogram places the Piroplasmida (Babesiidae and Theileriidae) as a monophyletic unit, and *Theileria* monophyletic with *Cytauxzoon*. This agrees with previous systematic work. Interestingly, *Cryptosporidium* falls outside the order Eimeria and into an unusual, but weakly supported, monophyletic grouping of Order Eucoccidiorida and Order Haemosporidia from the class Gregarina. The order Eimeriida is monophyletic (with the exception to the *Cryptosporidium* previously noted). The families Eimeriidae and Sarcocystidae show as sister clades with the exception to the Eimeriidae *Isospora* placement within the Sarcocystidae. The two cladograms represented are identical in methodology with the exception to Figure 23a includes gaps and Figure 23b excludes gaps from analyses. With gaps excluded, the bootstrap values are significantly higher, and the topologies nearly identical.

Table 9. Current accepted systematics for Apicomplexa for taxa used in this study using NCBI nomenclature (www.ncbi.nlm.nih.gov).

- Kingdom Protista
  - Phylum Apicomplexa
    - Class Coccidia
      - Order Eimeriida
        - Family Cryptosporidiidae
          - Genus *Cryptosporidium*
        - Family Eimeriidae
          - Genus *Caryospora*
          - Genus *Cyclospora*
          - Genus *Eimeria*
          - Genus *Isospora*
        - Family Sarcocystidae
          - Genus *Hammondia*
          - Genus *Hyaloklossia*
          - Genus *Neospora*
          - Genus *Sarcocystis*
          - Genus *Toxoplasma*
      - Order Eucoccidiorida
        - Family Haemogregarinidae
          - Genus *Hepatozoon*
          - Genus *Haemogregarina*
        - Family Lankesterellidae
          - Genus *Lankesterella*
        - Family Colpodellidae
          - Genus *Colpodella*
    - Class Gregarina
      - Order Eugregarinida
        - Family Gregarinidae
          - Genus *Gregarina*
        - Family Leidyanidae
          - Genus *Leidyana*
        - Family Monocystidae
          - Genus *Monocystis*
        - Family Ophryocystidae
          - Genus *Ophryocystis*
        - Family Pseudomonocystidae
          - Genus *Pseudomonocystis*
      - Order Haemosporidia
        - Family Plasmodiidae
          - Genus *Plasmodium*
    - Class Piroplasmida
      - Family Babesiidae
        - Genus *Babesia*
      - Family Theileriidae
        - Genus *Cytauxzoon*
        - Genus *Theileria*

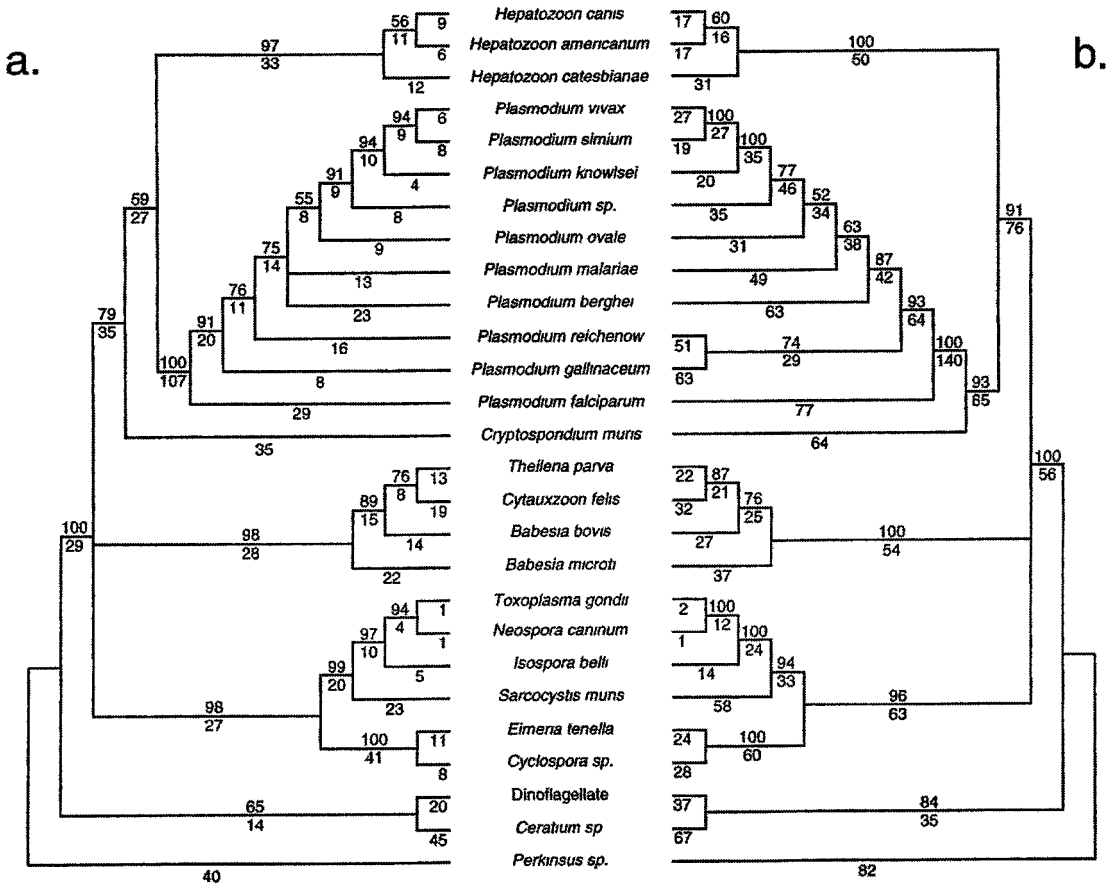


Figure 23. Phylogenetic hypotheses of Apicomplexa based on DNA sequence variation of the 18S rRNA gene. **a**. Results of bootstrap analysis with equal weights applied to transitions and transversions and gap positions included in the analysis. Numbers above each clade represent the percentage of bootstrap iterations in which each clade was detected, whereas numbers below each lineage represent branch lengths. **b**. Results of bootstrap analysis with equal weights applied to transitions and transversions and gap positions excluded from the analysis. Numbers above each clade represent the percentage of bootstrap iterations in which each clade was detected, whereas numbers below each lineage represent branch lengths Entire diagram and legend from Mathew et al. (2000) as comparison of previously accepted molecular phylogenetic tree.

It is important to examine any phylogenetic hypotheses meticulously including methods of tree construction. In phylogenetic analyses, there are currently three main techniques for deducing the true tree: maximum parsimony, distance methods, and maximum likelihood. These three methods of tree reconstruction utilize different criteria for selecting the best estimate of the true evolutionary history. Different models can be

compared only by how well they fit the data with respect to a particular optimization criterion. The best fitting tree is that which maximizes the signal in the data set and minimizes the homoplastic variation. For maximum parsimony, the best tree is that which describes the minimum sequence evolution with the shortest treelength. For distance methods, the best tree is that which minimizes total sum branch length changes with the lowest minimum evolution (ME) score. For maximum likelihood, the best tree is the tree which has the best likelihood of the data fit with the model of evolution which is the lowest -lnL score (or the highest probability of the data fit). It should be noted, however, that the tree with the optimal score is not necessarily the true tree (Takahashi & Nei, 2000).

Maximum parsimony is the principle that aims to minimize the number of evolutionary changes required to explain the distribution of the data on the tree. This theory abides by Ockham's Razor in which the best hypothesis is the one requiring the smallest number of assumptions (Graur & Li, 2000). Optimal trees therefore have the shortest treelength in which the smallest number of character changes is required. The hypotheses of evolutionary relationships do not necessarily assume that evolution always occurs parsimoniously, however parsimony is used as a method for choosing between competing evolutionary relationships (Takahashi & Nei, 2000). This method simply assumes that apparent homology is more likely attributable to true homology than homoplasy, and follows that one change on one branch is more likely than two on different branches (Mishler, 1994). By minimizing the number of substitutions (or steps), this reduces the number of homoplasies (parallel, convergent, and back

substitutions). The advantages of maximum parsimony include its minimal assumptions about character state change and for small data sets (< appx. 13 taxa) can often find a globally optimal solution by full searches (Swofford et al., 1996). One disadvantage of parsimony includes its production of sets of equally short parsimonious trees which can differ distinctly in topologies. These trees may have identical tree statistics and therefore no one tree can be supported. Another disadvantage of maximum parsimony is that it is limited by an underestimation of branch lengths for sequences that have undergone these homoplastic substitutions.

Distance matrix methods consider the evolutionary distances (i.e., the number of nucleotide substitutions between two taxa) for all pairs of taxa and constructs a phylogenetic tree using an algorithm based on a clustering criterion among the distance values. There are several reasons that distance characters are superior to character data. First, DNA sequence data is more informative if converted into a mathematical comparison in the similarities/differences in the sequences. Second, multiple substitutions at one site can be corrected for by the comparison of the number of substitutions between two sequences however correction methods would not apply to the individual sequences. Third, there are many distance methods as well as distance correction algorithms that are fast and efficient even for large numbers of taxa (Graur & Li, 2000). The distance measure of neighbor-joining finds the shortest tree by sequentially clustering neighbors that minimize the total number of changes on the tree. A main disadvantage of distance methods during the conversion of character data into distance data information can be lost to allow conversion back to the character data

(Swofford et al., 1996). Another disadvantage is that there is no biological consideration for clustering nor does it lead to identification of highly informative characters. The advantages of distance methods include a single solution and its relative fast computational time.

Maximum likelihood is the method that evaluates the probability of observing the data under a specific topology and a specified model of character state changes or substitution model. The likelihood is evaluated by a logarithmic function or the log likelihood (ln L) of the tree. The maximum likelihood tree is therefore the tree with the highest likelihood value (or higher probability for the observed state). The probabilities of rate substitution are dependent upon the assumptions about how the nucleotides evolved and evolutionary time. It is therefore possible to have a tree with the highest likelihood value under one substitution model not be the same maximum likelihood tree under a different model of nucleotide substitution (Graur & Li, 2000). The advantages of maximum likelihood include its tendency to be consistent, to have low variance, and it tends to outperform alternative methods when evaluated under many models of sequence evolution (Swofford et al., 1996). The disadvantage includes its increased number of assumptions about character evolution that could be incorrect.

The molecular clock hypothesis proposes that for a sequence the rate of substitution is approximately constant over time in all evolutionary lineages and that the rate of evolution is equal to the rate of mutation (Li, 1997). Substitution rate differences could arise in sequences with an unbalanced transition and transversion ratio as well as

homoplastic substitutions (parallel, convergent, and back substitution). Transitions (purine → purine, or pyrimidine → pyrimidine) are found to occur more frequently than transitions (purine → pyrimidine, or pyrimidine → purine) due to an energetic difference in the reaction. Back substitutions (A→ T → A) would be expected over evolutionary time since there are only four possible character states and some sites will have reverted back to their original state (Mindell & Thaker, 1996). In phylogenetic analyses, it is essential to consider these frequency differences and reversions in order to accurately reconstruct phylogenetic relationships.

The number of nucleotide substitutions between two sequences can be used to compute the rate of evolution, to estimate divergence time, and can be used in reconstructing phylogenetic trees (Yang, 1996). By comparing two sequences that share apomorphic characters, it is reasonable to consider that a single nucleotide substitution may take millions of years to occur. In considering sequence divergence, one must consider the case of multiple substitutions at the same site. The impact of multiple substitutions is the number of observed substitutions would be less than the actual number of substitutions. After the separation of two nucleotide sequences, each sequence will accumulate their own substitutions which increase divergence between the two sequences. Over a significant period of time, however, the probability of multiple substitutions at the same site increases, which will decrease rather than increase the differences between the two sequences (Li, 1997). These multiple substitutions at the same site may lead to parallel substitutions, coincidental substitutions, convergent substitutions, and back substitutions. One must also consider that there are portions of

sites that are unable to allow substitutions (possibly due to a strong functional constraint), however the remaining sites would continue to vary at a particular rate (Swofford et al., 1996). These factors must be considered when choosing an appropriate phylogenetic method in attempt to correct for homoplasy.

It is possible to correct for these nucleotide substitution anomalies by choosing a nucleotide substitution model that differs in the assumptions about the process of nucleotide substitutions. It is expressed as substitutions per site per evolutionary time at which each nucleotide is replaced by each alternative nucleotide. PAUP* has the twelve most commonly used correction algorithms, however there are over 50 possible correction algorithms used today. Distance correction methods correct unequal base frequencies and/or unequal substitutions that may be a source saturation in the dataset. Tajima-Nei (1984) also assumes that all there are unequal base frequencies and only one substitution type. The general time reversible (GTR) from Lanave et al. (1984) and Rodriguez et al. (1990) assumes unequal base frequency and 6 substitution types. Tamura-Nei corrects for unequal base frequencies and 3 substitution types (Tamura & Nei, 1981). HKY85 corrects for unequal base frequencies and unequal Ti:Tv ratio (Hasegawa et al., 1985). One can either have PAUP* calculate the base frequency percentages or use another software program such as Modeltest (v.3.06, Posada 2001) to calculate the data set statistics to choose between nucleotide substitution models. Modeltest uses log likelihood scores to establish the model of DNA evolution that best fits the data and produces output that is specific to the data set to be used in maximum likelihood analyses.

There are several confidence measures for assessing the reliability of a particular data set: character support, branch support, and topology support. Character support can be determined by the consistency index, retention index, and rescaled consistency index provided in PAUP*. These values range from 0 for homoplastic characters to 1 for consistent characters.

Branch support can be evaluated by the bootstrap and jackknife methods that provide pseudoreplicates within the data set. The bootstrap technique provides statistical confidence by repeatedly sampling data from the original sample data set. Sampling with replacement means that a sampled site can be resampled with the same probability as any other site (Felsenstein, 1985), therefore some sites may be sampled multiple times and some sites may never be sampled. The bootstrap value is expressed as the number of replicates that support a particular clade, where 100% is the highest support. Jackknifing is another resampling technique which is very similar to bootstrapping, however eliminates the chance of resampling the same site. Jackknifing randomly takes a certain percentage (usually 50%) of the original data set and creates a new matrix and new tree, and this process is repeated usually 1,000 times. The jackknife value is also expressed as the number of replicates that support a particular clade (Graur & Li, 2000).

Topology support can determine if there is phylogenetic signal in the data set, and whether a topology is significantly favored over another topology. Congruent trees are derived from different data sets or methodologies but reveal identical topologies. The

congruence of topologies supported by independent data partitions is considered some of the strongest support for phylogenetic relationships (Cunningham, 1997). If the data set has undergone the same evolutionary history, then incongruity between data partitions must occur from phylogenetic inaccuracy (Bull et al., 1993). It has also been shown that congruence is valuable in parsimony when it is uncertain when to apply weighting methods. Congruence between two topologies in which the data has been analyzed by different methodologies further supports a given topology in a data set.

Phylogenetic analyses are essential in the classification of organisms and use synapomorphic characters to group similar taxa. There are two central concepts used to guide attempts to classify organisms into naturally occurring "species." The Phylogenetic (Evolutionary) Species concept contends that a species is "a single lineage of ancestral-descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate (Wiley, 1978). Under this theory, species are defined as the smallest diagnosable monophyletic group. Cladogenesis on a phylogenetic tree represents a distinguishable amount of dissimilarity between taxa that once shared a common ancestor. This differs in philosophical basis from the Biological Species Concept which considers species valid only when a group of individuals are capable of exchanging genetic material with each other but remain reproductively isolated from all other such groups (Mayr, 1942). This theory does not allow for hybridization between "species." Both species concepts are limited with respect to convergence in morphological and molecular data.

The widely distributed reports of the occurrence of *Haemogregarina* in turtles prompted the investigation of all accessible populations for the current study. This includes endemic Texas turtle genera from several localities as well as translocated Asian turtles under captive management. In an effort to evaluate the phylogeny, taxonomy, host specificity, and co-evolutionary status of *Haemogregarina* in naturally-occurring populations and translocated populations, the following objectives were delineated:

1) Generate molecular sequence for *Haemogregarina* utilizing novel primers specific to the parasite and exclusive of the turtle host;

2) Align *Haemogregarina* sequences with all available Apicomplexa 18S molecular sequence for maximum sequence homology and minimal gaps;

3) Determine the phylum-level apicomplexan topology and placement of *Haemogregarina*;

4) Evaluate the family-level topology of Haemogregarinidae to determine systematics of *Haemogregarina* from different turtle hosts; and,

5) Assess the host specificity and coevolutionary status for *Haemogregarina* for different populations of turtles.

METHODS

Alignment of sequences

All Apicomplexa sequences were obtained from NCBI's GenBank nucleotide database (http://www.ncbi.nlm.nih.gov/), imported into Sequencher (v.4.12, GeneCodes Corp.) and aligned to determine sequence homology (See Phillips et al., 2000). For all apicomplexans, only the 18S gene of nDNA was chosen due to its evolutionary studies on this phylum previously (Morrison & Ellis, 1997; Smith et al., 1999; Mathew et al., 2000; Perkins & Keller, 2001). Table 10 lists the genera and their corresponding GenBank accession numbers and length in base pairs prior to alignment. All organisms are Apicomplexans with the exception to *Entamoeba histolytica* which was chosen for an outgroup to the apicomplexans. GenBank does not currently contain sequence for *Haemogregarina*. When available, multiple representatives from a taxa were used in attempt to avoid the prospect of chance convergence that can occur due to only four character states in nucleotide sequence (Hedges et al., 1990).

Once the apicomplexan sequences were aligned using Sequencher software, reptilian taxa (18S) were included to ensure primer specificity to the parasite and excluding the host. The reptilian taxa and GenBank accession numbers are as follows: *Alligator mississippiensis* [AF173605], *Heterodon platyrhinos* [M59392], *Sceloporus undulatus* [M59400], and *Trachemys scripta* [M59398]. Previous molecular analyses primers were also included in the alignment to determine affinity to vertebrate host and

apicomplexan parasite. Novel primers were developed based on this alignment and used for further molecular analyses to amplify *Haemogregarina* sp. from infected turtles that were diagnosed from the corresponding blood smears. For the laboratory

Table 10. Table of sequences used for alignment for 18S nrDNA obtained from GenBank (NCBI) with the accession number and the length in base pairs noted. Sequences were aligned in Sequencher (v.4.12,GeneCodes Corp.) and examined for development of novel primers to amplify *Haemogregarina* (sequences not available through GenBank) during Polymerase Chain Reaction amplification. The length of each taxa is represented as the number of bases prior to additional gaps required for alignment.

Organism	GenBank No	Length
Babesia divergens	U16370	1724
Babesia odocoilei	U16369	1723
Caryospora bigenetica	AF060975	1751
Caryospora bigenetica	AF060976	1751
Colpodella pontica	AY142092	1779
Colpodella sp	AY142075	1730
Cryptosporidium sp	AF112573	1743
Cryptosporidium felis	AF112575	1781
Cryptosporidium meleagridis	AF112574	1744
Cryptosporidium parvum	AF161859	1746
Cryptosporidium parvum	AF161858	1746
Cryptosporidium parvum	AF112576	1741
Cryptosporidium parvum	AF112572	1749
Cryptosporidium parvum	AF164102	1746
Cryptosporidium parvum	AF112571	1750
Cryptosporidium parvum	AF112569	1748
Cryptosporidium parvum	AF112570	1750
Cryptosporidium parvum	AF115377	1749
Cryptosporidium parvum	L25642	1507
Cryptosporidium wrairi	AF115378	1746
Cyclospora colobi	AF111186	1795
Cyclospora papionis	AF111187	1796
Cytauxzoon felis	L19080	1774
Cytauxzoon sp	AF531418	1485
Eimeria falciformis	AF080614	1778
Eimeria weybridgensis	AY028972	1741
Entamoeba histolytica	X65163	1947
Entamoeba histolytica	X89636	1162

Organism	GenBank No	Length
Gregarina caledia	L31799	1210
Gregarina chortiocetes	L31841	1210
Hammondia hammondi	AH008381	1747
Hepatozoon catesbianae	AF176837	1120
Hepatozoon canis	AF206669	368
Hepatozoon canis	AF176835	1120
Hepatozoon sipedon	AF206671	367
Hepatozoon americanum	AF176836	1413
Hepatozoon sp	AF418558	625
Hyaloklossia lieberkuehni	AF298623	1564
Isospora robini	AF080612	1790
Isospora suis	U97523	1822
Leidyana migrator	AF457130	1461
Lankesterella minima	AF080611	1781
Monocystis agilis	AF213514	793
Monocystis agilis	AF457127	1773
Neospora caninum	U17346	1747
Ophriocystis elektroscirrha	AF129883	1811
Perkinsus atlanticus	U07697	60
Perkinsus olseni	U07701	60
Plasmodium berghei	AJ243513	2067
Plasmodium vivax	U93095	2330
Pseudomonocystis lepidi	L31843	1196
Sarcocystis sp	U97524	1894
Sarcocystis tenella	L24383	1782
Theileria sp	U97056	1750
Theileria parva	L02366	1742
Toxoplasma gondii	L37415	1738
Toxoplasma gondii	L24381	1723

techniques and methods used to generate DNA sequences included in the phylogenetic analyses of *Haemogregarina*, see Chapter 2.

For all phylogenetic analyses, the computer software PAUP* (v4.0b10, Swofford, 2002) and MacClade (Maddison & Maddison, 1992) were utilized. Three datasets for the

Apicomplexa were chosen because of the disparity in sequence length to avoid erroneous homology and to limit the number of uninformative gaps. The first dataset (A) consisted of 1075 characters, however only had 46 taxa. Dataset B had only had 463 characters, however contained all the taxa (n=63) including *Haemogregarina*. The third dataset (C) consisted of *Haemogregarina* sequences and outgroups (n=16) in attempt to resolve the genera-level synapomorphies. All three molecular datasets for the apicomplexans (A through C) and the one dataset for the turtles (D) were analyzed using the same methods unless otherwise stated.

Final alignment for all Apicomplexa data sets:

- see Appendix C for alignment of all Apicomplexa
- included vertebrates to ensure novel primer specificity only to Apicomplexa
- totaled 74 taxa

Dataset A:

- totaled 46 taxa and length of 1075 characters (see Figure 24)
- Sequences that did not 100% complete the predetermined required length (based on sequence length of AF176835 and AF176837 *Hepatozoon*) were removed:
    - All 4 vertebrate taxa
    - U07697 *Perkinsus atlanticus*, U07701 *Perkinsus olseni*
    - All 13 *Haemogregarina* sp.
    - AF418558 *Hepatozoon* sp., AF206669 *Hepatozoon canis*, AF206671 *Hepatozoon sipedon*
    - AF213514 *Monocystis agilis*

- L31799 *Gregarina caledia*, L31841 *Gregarina chortiocetes*
- X89636 *Entamoeba histolytica*
- AF531418 *Cytauxzoon* sp.
- L31843 *Pseudomonocystis lepidi*

- region is from base 193 to 1642 of final alignment (Appendix C) with the following regions removed due to large gaps inserted due to either autapomorphies or a few taxa containing insertions:
  - 224-231, 269-301, 357-373, 441-457, 539-548, 653-662, 788-996, 1049-1064, 1361-1415, 1643-2459.

Dataset B:

- totaled 63 taxa and length of 463 characters (see Figure 24)
- Sequences that did not 100% complete the predetermined length (based on sequence length of MF5494 *Haemogregarina* sp.) required were removed:
  - All 4 vertebrate taxa
  - U07697 *Perkinsus atlanticus*, U07701 *Perkinsus olseni*
  - AF213514 *Monocystis agilis*
  - MF5510 *Haemogregarina* sp.
- region 1260 to 2056 of final alignment (Appendix C) with the following regions removed due to excess gaps:
  - 1361-1415
  - 1807-1997

Dataset C:

- totaled 16 taxa and length of 522 characters (see Figure 24)
- region 1260 to 2056 of final alignment (Appendix C) with gapped regions removed:
    - 1361-1415
    - 1807-1997
- the two *Hepatozoon canis* sequences were consensed for a *Hepatozoon.canis.consensus* sequence in order to fulfill 100% integrity of the dataset.
- the *Hepatozoon catesbianae* and *Hepatozoon sipedon* sequences were determined to be phylogenetically closest with respect to all other *Hepatozoon* and therefore combined to provide a full length sequence providing no missing data in the final datset.
- Sequences that did not 100% complete the predetermined length (based on sequence length of MF5494 *Haemogregarina* sp.) required were removed. This included the same taxa removed as in Dataset B.

Dataset D:

- totaled 8 taxa and length of 966 characters of partial ND4-Hist-Ser-Leu isolated from the mitochondrial genome.

- contained turtles that were hosts to the apicomplexan parasites. In the case where the turtle genera sequence was not available, a phylogenetically similar genera was used due to time constraints.
- evaluated for congruence with Apicomplexan trees to determine similarity resulting from coevolution.
- See appendix E for alignment

Phylogenetic analyses

For all phylogenetic analyses, all molecular characters were weighted equally. In the Apicomplexa datasets A and B the two *Entamoeba histolytica* taxa were set as monophyletic sistergroup to the ingroup. For the *Haemogregarina* sp. dataset, *Monocystis* and *Ophryocystis* were set as monophyletic sistergroup to the ingroup. For the turtle dataset D, *Trachemys scripta* was set as outgroup rooted at internal node with basal polytomy. The following methods were performed for datasets A through D:

In order to determine if there was phylogenetic signal in the dataset, PAUP* analyzed 10,000 random trees. The frequency distribution of treelengths was graphed in Excel to evaluate left-skewness which illustrates the phylogenetic signal for the dataset. The mean treelength, standard deviation, g1 statistic, and g2 statistic were recorded. In order to determine if the base frequencies for the dataset were equal or unequal, the base frequencies were given as totals for all taxa in the dataset.

To determine if there is an unequal substitution pattern of transitions and transversions in the dataset, three analyses were performed. First, the maximum parsimony treefile of the first (random) tree was imported into MacClade and the "State Change & Stasis" substitution pattern was examined. Second, a maximum likelihood heuristic search was executed in PAUP* with equal base frequencies, equal among-site variation, and Ti/Tv ratio set to "Estimate." After one tree was obtained, the search was aborted and under maximum likelihood options selected "previous" which therefore gives the estimated Ti/Tv ratio. Third, with PAUP* set to distance optimality criterion under neighbor joining distances, the DNA/RNA distance was set to "uncorrected 'p.'" For the substitution restrictions, the three distances of all substitutions, transitions only, and transversions only were individually calculated and exported. The "transitions" and "transversions" were graphed in Microsoft Excel XP with "all substitutions" as the x-axis to determine saturation in the dataset.

For maximum parsimony analyses, the trees were rooted with respective outgroups in PAUP*. All characters were equally weighted and all gaps were treated as a 5^{th} base and then also as missing data. A heuristic search with ACCTRAN character state optimization for 1000 random replicates by stepwise addition with TBR branch swap was performed. A 50% bootstrap was then performed for 2500 replicates by full heuristic search with random stepwise additions for 1 random replicate. A 50% deletion jackknife search was also performed for 2500 replicates by neighbor joining search.

For distance analyses, the trees were rooted with respective outgroups in PAUP*. All characters were equally weighted and all gaps were treated as missing data. Based on the base frequencies and Ti/Tv ratios performed initially for each dataset, a distance correction model was chosen. A neighbor joining search was performed with the appropriate distance model. A 50% bootstrap was then performed for 2500 replicates by neighbor joining search. A 50% deletion jackknife search was also performed for 2500 replicates by neighbor joining search.

For maximum likelihood analyses, the datasets were evaluated using the Modeltest v3.06 software (Posada, 2001) to determine the appropriate model of nucleotide evolution that fits the respective dataset. The dataset was executed in PAUP*, and in the command line at the bottom of the display buffer typed "default lscores longfmt=yes" and then executed again. The "modelblock3" from the Modeltest examples file was then executed which began the testing of 56 different models of nucleotide substitution. After all 56 models were evaluated, the "model.scores" file was executed in Modeltest and the two most appropriate models were identified. The model selected, base frequencies, substitution model, and gamma for among site variation were also reported. These estimates were then executed in PAUP* for subsequent parameters in maximum likelihood analyses.

With the optimality criterion set to maximum likelihood, the Modeltest information was input and with a heuristic search with starting trees obtained via neighbor joining and TBR branch swap was executed and run until completion. See

Results section for Modeltest parameters for each dataset. A 50% bootstrap and a 50% deletion jackknife search was also performed for 2500 replicates by neighbor joining search.

RESULTS

Multiple datasets

Figure 24 depicts an overview of Apicomplexa 18S rDNA alignment and an outline of each dataset A, B, and C. Dataset A contains 46 taxa and length of 1075 characters; Dataset B contains 63 taxa and 463 characters; Dataset C contains 16 taxa and 522 characters. This overview contains all taxa used for the alignment including vertebrates and short sequences that were removed prior to phylogenetic analyses.

Table 11 details a summary of information for Apicomplexan and turtle datasets. All analyses were performed using PAUP* and MacClade. This information was used to determine particular phylogenetic analyses for an accurate phylogeny. For the Apicomplexa phylum-level analyses, Dataset A contains less taxa but more characters whereas Dataset B has more taxa with fewer characters. Dataset C is the smaller dataset of *Haemogregarina* sequences. Dataset D (turtles) is the partial ND4-His-Ser-Leu region of the mitochondrial genome. All four datasets reveal an unequal base frequency ($\neq$ 25%). The transition to transversion ratio for datasets A, B, and C reveal that there is only one substitution type (Ti = Tv), however for the turtle dataset D the Ti to Tv ratio is

2 to 1. The estimation of 10,000 random trees gives an approximation of the phylogenetic signal for each dataset. The degree of left skewness (not shown) or greatest negative number of the g1 statistic reveals a greater amount of signal in the dataset. For these analyses, the turtle dataset appears to have the greatest phylogenetic signal.

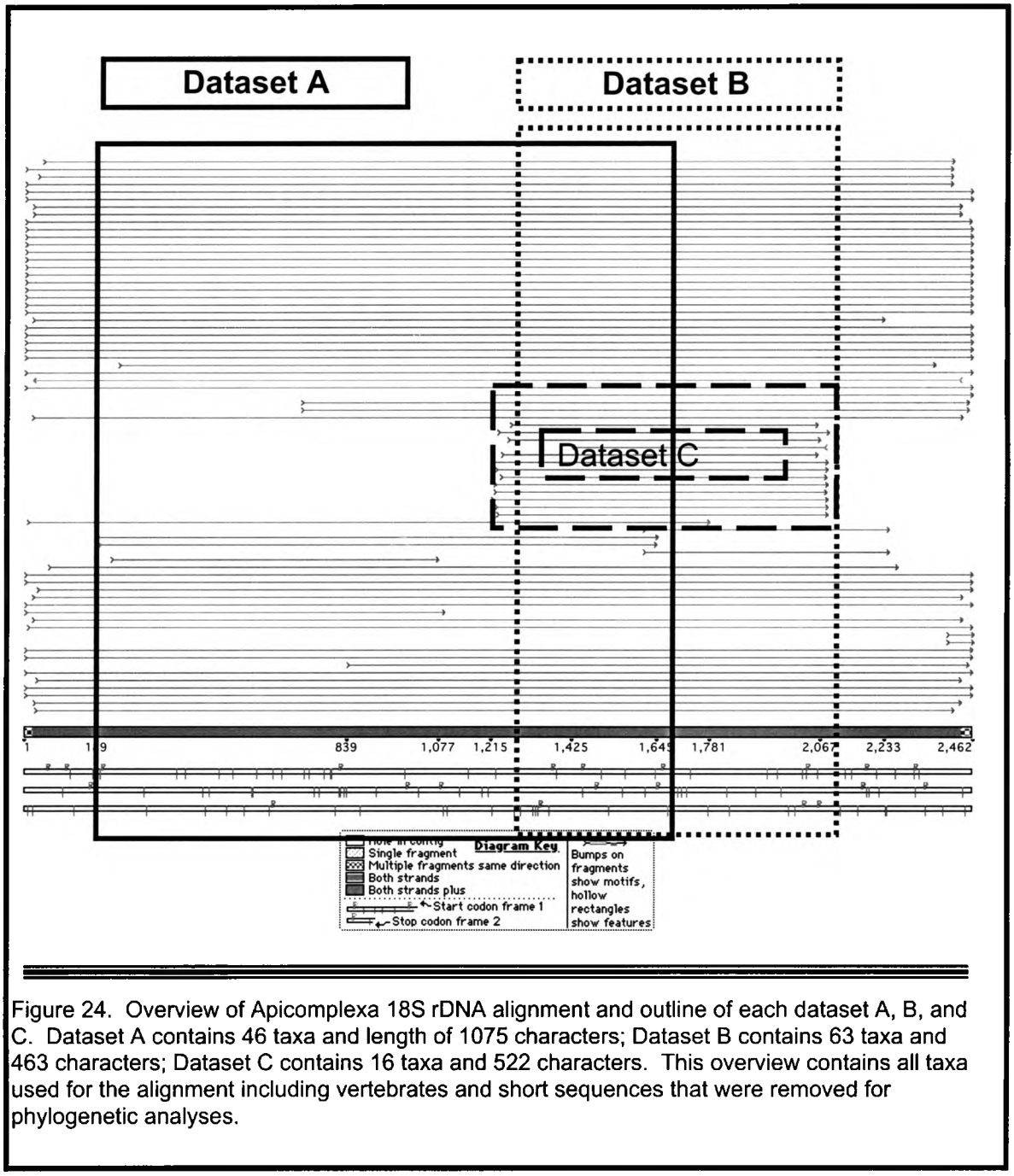


Figure 24. Overview of Apicomplexa 18S rDNA alignment and outline of each dataset A, B, and C. Dataset A contains 46 taxa and length of 1075 characters; Dataset B contains 63 taxa and 463 characters; Dataset C contains 16 taxa and 522 characters. This overview contains all taxa used for the alignment including vertebrates and short sequences that were removed for phylogenetic analyses.

Table 11 Summary of information for Apicomplexan and turtle datasets All analyses were performed using PAUP* (v4 0b10, Swofford, 2002) and MacClade (v 3 05, Maddison & Maddison, 1992) This information was used to determine particular phylogenetic analyses for an accurate phylogeny

	Dataset A	Dataset B	Dataset C	Dataset D
# of taxa	46 taxa	63 taxa	16 taxa	8 taxa
# of characters	1075 chars	463 chars	522 chars	966 chars
# of gaps	434 gaps	135 gaps	15 gaps	71 gaps
# uninformative	663 chars	288 chars	488 chars	715 chars
base frequencies				
A	30 483%	27 742%	27 715%	35 817%
C	17 748%	18 596%	18 389%	25 554%
G	24 879%	25 802%	26 005%	13 228%
T	26 890%	27 861%	27 891%	25 401%
Ti Tv ratio	571 549	231 245	33 29	277 136
Random trees				
mean treelength	3449 1717	1654 325	143 34	860 2392
sd	85 724164	35 0285	5 7201	34 7301
g1 statistic	-0 437967	-0 5676	-1 835842	-1 351147
g2 statistic	0 258079	0 65387	5 183469	1 779563

Figures 25 through 28 show the uncorrected pairwise distance for Datasets A through D respectively. Transitions are represented as dark colored diamonds and transversions are represented as light colored squares. Trendlines were added to view the point of saturation in the dataset where the trendlines intersect. Saturation is homoplasy due to multiple substitutions at an individual site.

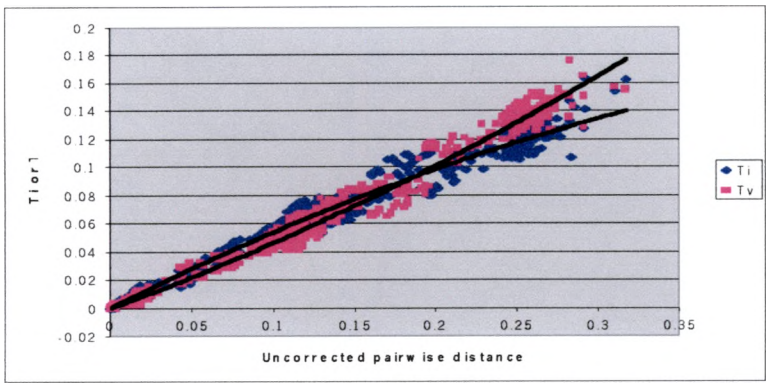


Figure 25. Uncorrected pairwise distance for Dataset A. Transitions are represented as dark colored diamonds and transversions are represented as light colored squares. Trendlines were added to view the point of saturation in the dataset where the trendlines intersect.

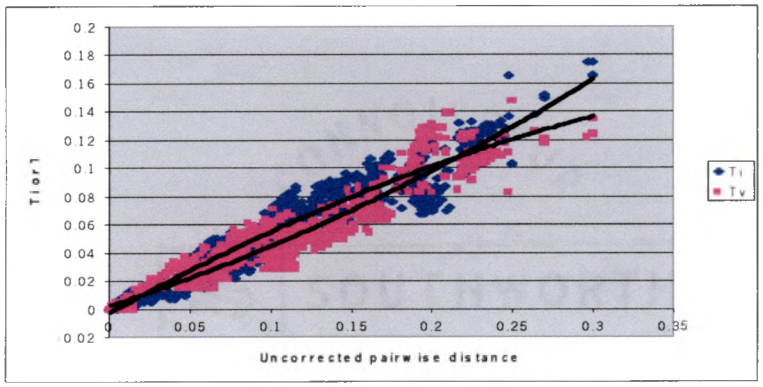


Figure 26. Uncorrected pairwise distance for Dataset B. Transitions are represented as dark colored diamonds and transversions are represented as light colored squares. Trendlines were added to view the point of saturation in the dataset where the trendlines intersect.

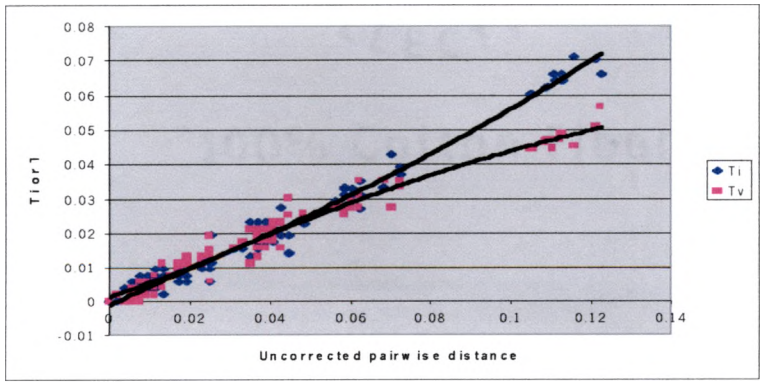


Figure 27. Uncorrected pairwise distance for Dataset C. Transitions are represented as dark colored diamonds and transversions are represented as light colored squares. Trendlines were added to view the point of saturation in the dataset where the trendlines intersect.

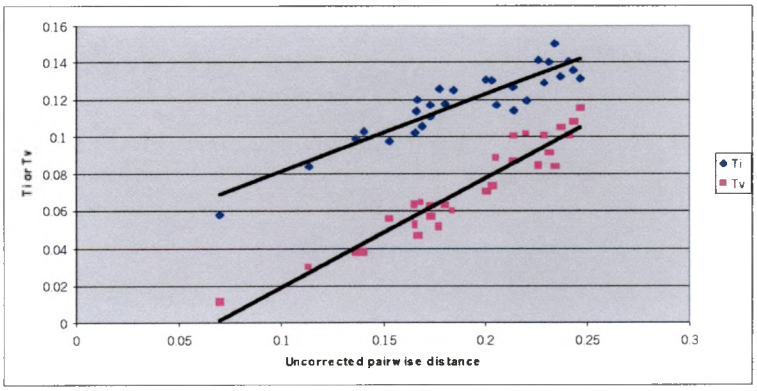


Figure 28. Uncorrected pairwise distance for Dataset D. Transitions are represented as dark colored diamonds and transversions are represented as light colored squares. Trendlines were added to view the point of saturation in the dataset where the trendlines intersect.

Table 12 Summary of tree statistics for phylogenetic analyses for phylum Apicomplexa (Datasets A &B), family Haemogregarinidae (Dataset C) , and turtle hosts (Dataset D)

	Dataset A	Dataset B	Dataset C	Dataset D
Max Parsimony (gaps 5th base)				
#MP trees	2	95	55	1
TL	2749	1004	136	786
CI	0 536	0 520	0 882	0 763
RI	0 698	0 769	0 800	0 582
RC	0 374	0 400	0 706	0 444
Max Parsimony (gaps missing)				
#MP trees	30	79	81	1
TL	1796	695	116	707
CI	0 579	0 557	0 862	0 748
RI	0 734	0 787	0 787	0 571
RC	0 425	0 438	0 678	0 427
Neighbor Joining				
ME score	1 64636	1 39749	1 39749	0 75164
Max Likelihood				
Model 1 -lnL	9273 59391	3774 12108	1387 16836	4161 10413
Model 2 -lnL	9271 50200	3770 59511	1338 86140	n/a

Table 12 shows the summary of tree statistics for phylogenetic analyses for Datasets A, B, C and D. The best fitting tree is that which maximizes the signal in the data set and minimizes the homoplastic variation. For maximum parsimony, the best tree is that which describes the minimum sequence evolution with the shortest treelength. The character support indices (CI, RI, RC) range from 0 for homoplastic characters to 1 for consistent characters. For distance methods, the best tree is that which minimizes total sum branch length changes with the lowest minimum evolution (ME) score. For maximum likelihood, the best tree is the tree which has the best likelihood of the data fit with the model of evolution which is the lowest -lnL score (or the highest probability of

the data fit). It should be noted, however, that the tree with the optimal score is not necessarily the true tree (Takahashi & Nei, 2000).

Description of cladograms and phylograms in Appendix F:

For an exhaustive reconstruction of phylogenies for the various datasets, the extensive number of trees are in Appendix F. The trees are discussed in the current results section and the final topology for each dataset is presented in the discussion for this chapter.

Dataset A:

Maximum parsimony with gaps as 5th base:

Figures F-1 and F-2 in Appendix F show the 2 most parsimonious trees with gaps were treated as a 5th base. The 2 most parsimonious trees had a TL=2749, CI=0.536, RI=0.698, and RC=0.374. The two most parsimonious topologies differ only in the placement of the *Cryptosporidium parvum* isolates L25642 and AF112569 within the monophyletic Cryptosporidiidae. *Leidyana migrator* failed to demonstrate monophyly within the Eugregarinida order, and the *Isospora suis* failed to demonstrate monophyly within the Eimeriidae with the other *Isospora* isolate. *Lankesterella* shows monophyly within the Eimeriidae and fails to exhibit placement as its own family within Eucoccidiorida. *Cytauxzoon* fails to show monophyly within the Theileriidae.

Figures F-3 and F-4 in Appendix F shows the bootstrap consensus and jackknife consensus trees by maximum parsimony with gaps were treated as 5th base. Bootstrap and jackknife values <50% support are not shown. For both topologies, the class Gregarina fails to demonstrate monophyly, and the class Coccidia also fails to exhibit monophyly. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiididae) show strong support as monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in Figures F-1 and F-2. The systematically established monophyly of *Cytauxzoon* within the Theileriidae shows stronger support for jackknife consensus in Figure F-4 than on the bootstrap consensus from Figure F-3.

Maximum parsimony with gaps as missing base:

Figures F-5 and F-6 show the strict consensus and majority rule consensus respectively for 30 most parsimonious trees with gaps treated as missing. The 30 most parsimonious trees had a TL=1796, CI=0.579, RI=0.734, and RC=0.425. In **Figure F-5** for the strict consensus, the families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiididae) represent monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in Figures F-1 through F-4 with gaps treated as a 5th base under maximum parsimony criterion. The systematically accepted Eucoccidiorida (Hepatozoidae, Lankesterellidae, and Colpodellidae) fails to exhibit monophyly, as well as the Eugregarinidae

(Leidyanidae, Monocystidae, and Ophryocystidae) fails to demonstrate a monophyletic unit.

In **Figure F-6** for the majority rule consensus tree, this demonstrates that the topology differences among the 30 most parsimonious trees lie within the Cryptosporidiidae. All other branches on this topology show 100% of the same topology for the 30 most parsimonious trees. This topology differs to Figures F-1 and F-2 (gaps as 5th base) in the presence of monophyly of Theileridae, presence of polytomy with *Toxoplasma* and *Hammondia*, lack of monophyly within Eugregarinida, and minor differences within Cryptosporidiidae.

Figure F-7 shows the bootstrap consensus tree by maximum parsimony with bootstrap values <50% support not shown with gaps treated as missing. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiididae) show strong support as monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in all previous topologies. This bootstrap consensus shows stronger support than the bootstrap consensus from Figure F-3 with gaps as missing data.

Figure F-8 shows the jackknife consensus tree by maximum parsimony with gaps treated as missing data. Jackknife values with <50% support are not shown. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiididae) demonstrate monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in all previous topologies. This jackknife topology shows weaker support for classes within Apicomplexa however

resolves polytomies compared to the jackknife topology from Figure F-4 with gaps as missing data.

Neighbor-joining optimality criterion:

Figure F-9 shows the neighbor joining cladogram with optimality criterion set to distance with a search and Tajima Nei distance correction. The ME value is 1.64636. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiididae) demonstrate monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in all previous topologies. The Eugregarinida order fails to demonstrate monophyly, however the taxa within Cryptosporidiidae show resolution. **Figure F-10** shows the same topology as in Figure F-9, however the number of substitutions per site are represented as a phylogram.

Figures F-11 and F-12 show the bootstrap consensus tree and jackknife consensus tree respectively by neighbor joining with Tajima Nei distance correction algorithm. Bootstrap and jackknife values <50% support are not shown. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiididae) demonstrate monophyletic units respectively however show weak support. Eugregarinida fails to exhibit monophyly, and the Colpodellidae, Crytptosporidiidae, Hepatozoidae, and Class Gregarina collapse into a polytomy. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in all previous topologies.

Maximum likelihood optimality criterion:

Figure F-13 shows the maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model GTR + I + G; base frequencies of A=0.3265, C=0.1652, G=0.2313, T=0.2771; substitution rate matrix of A-C=1.2767, A-G=2.5466, A-T=1.8424, C-G=0.8904, C-T=4.8326, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.3531. The –ln L score is 9273.59391. The Cryptosporidiidae, Colpodellidae, Monocystidae, and Ophryocystidae form a monophyletic clade that disagrees systematically. *Leidyana* forms a monophyletic clade with *Colpodella* alternatively to its systematic order Eugregarinida. *Isospora suis* and *Lankesterella* continue in their disagree with systematic position.

Figure F-14 shows the bootstrap consensus for maximum likelihood with branch lengths shown above branches using Modeltest parameters. The parameters included model GTR + I + G; base frequencies of A=0.3265, C=0.1652, G=0.2313, T=0.2771; substitution rate matrix of A-C=1.2767, A-G=2.5466, A-T=1.8424, C-G=0.8904, C-T=4.8326, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.3531. Bootstrap values <50% support are not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa.

Figure F-15 shows the maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters for Akaike Information Criterion (AIC). The parameters included model GTR + I + G; base frequencies of A=0.3263, C=0.1647, G=0.2311, T=0.2778; substitution rate matrix of A-C=1.2873, A-G=2.5613,

A-T=1.8402, C-G=0.8976, C-T=4.8366, and G-T=1.0000; ASRV invariable sites = 0.1126 and variable sites with a gamma distribution of 0.4359. The –ln L score is 9271.50200. This maximum likelihood model compared to Figure F-14 (different Modeltest parameters) is identical in topology, however the branch lengths are slightly different.

Figure F-16 shows the bootstrap consensus of maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model GTR + I + G; base frequencies of A=0.3263, C=0.1647, G=0.2311, T=0.2778; substitution rate matrix of A-C=1.2873, A-G=2.5613, A-T=1.8402, C-G=0.8976, C-T=4.8366, and G-T=1.0000; ASRV invariable sites = 0.1126 and variable sites with a gamma distribution of 0.4359.

Dataset B:

Maximum parsimony with gaps as 5th base:

Figure F-17 shows the strict consensus of 95 most parsimonious trees with gaps treated as 5th base. The 95 most parsimonious trees had a TL=1004, CI=0.520, RI=0.769, and RC=0.400. The strict consensus collapses into multiple polytomies for most families of Apicomplexa. The order Eugregarinida fails to demonstrate monophyly, *Lankesterella* fails to group with the systematically accepted Eucoccidiorida, *Isospora suis* fails to group within Eimeriidae, and the families Sarcocystidae and Eimeriidae lack resolution.

The *Haemogregarina* and *Hepatozoon* genera group with the *Colpodella*, however collapse as polytomies within this clade.

Figure F-18 shows the majority rule consensus of 95 most parsimonious trees with gaps treated as 5th base. Majority rule values are represented above each branch length. The 95 most parsimonious trees had a TL=1004, CI=0.520, RI=0.769, and RC=0.400. The majority rule consensus shows that the 95 most parsimonious trees differ in the placement of taxa within Theileriidae, within Hepatozoidae, and within Eugregarinida.

Figures F-19 and F-20 show the bootstrap consensus tree and jackknife consensus tree respectively by maximum parsimony with gaps treated as 5th base. The bootstrap values with <50% support are not shown. The bootstrap consensus topology shows that the overall topology lacks support (bootstrap value = 50 and jackknife value = 51) and all major clades collapse into a polytomy.

Maximum parsimony with gaps as missing data:

Figure F-21 shows the strict consensus of 79 most parsimonious trees with gaps treated as missing data. The 79 most parsimonious trees had a TL=695, CI=0.557, RI=0.787, and RC=0.438. The strict consensus almost completely collapses the Sarcocystidae, combines Colpodellidae monophyletic with Cryptosporidiidae, and bifurcates the Eugregarinida. The placement of *Lankesterella*, and *Isospora suis*, show the same systematic problems as seen in all previous topologies.

Figure F-22 shows the majority rule consensus of 79 most parsimonious trees with gaps treated as missing. The 79 most parsimonious trees had a TL=695, CI=0.557, RI=0.787, and RC=0.438. Majority rule consensus values are represented above each

branch. This cladogram bifurcates the Eucoccidiorida families, bifurcates the Gregarina class, and bifurcates genera such as *Sarcocystis* and *Toxoplasma*.

Figures F-23 and F-24 show the bootstrap consensus tree and jackknife consensus tree respectively by maximum parsimony with gaps were treated as missing with bootstrap and jackknife values <50% support not shown. The topology has weak support for the first major bifurcation (bootstrap = 58 and jackknife =59) and most of the other clades collapse as polytomies.

Neighbor-joining optimality criterion:

Figure F-25 shows the neighbor joining cladogram with optimality criterion set to distance with a search and Tajima Nei distance correction algorithm. The ME value is 1.39749. This topology clusters Eucoccidiorida with Piroplasmida as a monophyletic unit. Sarcocystidae and Eimeriidae form a monophyletic clade, however the Gregarina class bifurcates among them. *Lankesterella* and *Isospora suis* are not grouped with their systematically approved classes. This cladogram bifurcates the Eucoccidiorida families, bifurcates the Gregarina class, and bifurcates genera such as *Sarcocystis* and *Toxoplasma*. **Figure F-26** is identical to Figure F-25, except the number of substitutions per site is represented as a phylogram.

Figures F-27 and F-28 shows the bootstrap consensus tree and neighbor joining consensus tree respectively by neighbor joining. Bootstrap and jackknife values <50% support not shown. These topologies show relatively strong support the first first bifurcation, however the majority of the clades collapse as polytomies. The

Hepatozoidae family is strongly supported and show monophyletic clades of *Haemogregarina* and *Hepatozoon* respectively. The placement of *Plasmodium* outside the Gregarina is weakly supported. *Toxoplasma* and *Sarcocystis* fail to group identical genera monophyletically.

Maximum Likelihood optimality criterion:

Figure F-29 shows the maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model TrN + G; base frequencies of A=0.3151, C=0.1648, G=0.2307, T=0.2894; substitution rate matrix of A-C=1.0000, A-G=1.9070, A-T=1.0000, C-G=1.0000, C-T=4.0781, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2339. The -lnL score is 3774.12108

Figure F-30 depicts the bootstrap consensus with maximum likelihood using Modeltest parameters. The parameters included model TrN + G; base frequencies of A=0.3151, C=0.1648, G=0.2307, T=0.2894; substitution rate matrix of A-C=1.0000, A-G=1.9070, A-T=1.0000, C-G=1.0000, C-T=4.0781, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2339. *Entamoeba histolytica* was set as outgroup to the Apicomplexa.

Figure F-31 shows the maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model TrN + G; base frequencies of A=0.2937, C=0.1655, G=0.2484, T=0.2924; substitution rate

matrix of A-C=1.6907, A-G=2.4927, A-T=1.5261, C-G=0.8853, C-T=5.1433, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2338. The -ln L score is 3770.59511. This topology is almost identical to Figure F-29 (different Modeltest parameters) yet differs in topology in the placement of the *Gregarina-Leidyana* monophyletic clade.

Figure F-32 shows the bootstrap consensus for maximum likelihood using Modeltest parameters. The parameters included model TrN + G; base frequencies of A=0.2937, C=0.1655, G=0.2484, T=0.2924; substitution rate matrix of A-C=1.6907, A-G=2.4927, A-T=1.5261, C-G=0.8853, C-T=5.1433, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2338.

Dataset C:

Maximum parsimony:

Gaps as 5th base:

Figure F-33 shows the strict consensus of 55 most parsimonious trees. *Monocystis agilis and Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. Gaps were treated as 5th base and nucleotides weighted equally. The 55 most parsimonious trees had a TL=136, CI=0.882, RI=0.800, and RC=0.706. All clades collapse as a polytomy with the exception to the TSE hosts.

Figure F-34 depicts a majority rule consensus of 55 most parsimonious trees with majority rule values represented above each branch. *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae. The 55 most parsimonious trees had a TL=136, CI=0.882, RI=0.800, and RC=0.706. This toplogy collapses into a polytomy with the exception to the TSE host clade, the HA-SC clade, and the PT-PN clade. *Hepatozoon* falls monophyletic within the *Haemogregarina* clade, thus not showing genera differentiation.

Figure F-35 shows the bootstrap consensus tree by maximum parsimony with bootstrap values <50% support not shown. This topology shows MF7952 *Haemogregarina* sp. (HG) is monophyletic with *Hepatozoon*, however this is weakly supported at bootstrap value of 57. The PT-PN clade is also weakly supported within *Haemogregarina*, however the TSE clade shows strong support.

Figure F-36 shows the jackknife consensus tree by maximum parsimony with jackknife values <50% support not shown. This topology shows MF7952 *Haemogregarina* sp. (HG) is monophyletic with *Hepatozoon*, however this is weakly supported at bootstrap value of 57. The PT-PN clade is not supported (as shown in Figure F-35) within Haemogregarina, however the TSE clade shows strong support.

Gaps as missing data:

Figure F-37 shows the strict consensus of 81 most parsimonious trees with gaps treated as missing data. The 81 most parsimonious trees had a TL=116, CI=0.862,

RI=0.787, and RC=0.678. The only distinguishable clade is the TSE-TSE monophyletic unit, and all other taxa form a polytomy.

Figure F-38 shows the majority rule consensus of 81 most parsimonious trees with gaps treated as missing data. The 81 most parsimonious trees had a TL=116, CI=0.862, RI=0.787, and RC=0.678. This topology groups *Haemogregarina* sp. (HG) as monophyletic with *Hepatozoon*. The majority of the trees bifurcate with the TSE-TSE clade and the PT-PN clade.

Figures F-39 and F-40 shows the bootstrap consensus and jackknife consensus trees by maximum parsimony with gaps treated as missing data and bootstrap and jackknife values <50% support not shown. The only strongly supported clade is the TSE-TSE monophyletic unit for both resampling methods.

Neighbor-joining:

Figures F-41 and F-42 show the cladogram and phylogram respectively with optimality criterion set to distance with a neighbor joining search and Tajima Nei distance correction algorithm. The resulting ME value is 0.21982. *Haemogregarina* (HG) forms a monophyletic clade with *Hepatozoon*. HT-HA-SC forms an Old World monophyletic unit, and PT-PN-SO-KS-TSE-TSE clade forms a New World monophyletic unit.

Figures F-43 and F-44 show the bootstrap consensus and jackknife consensus trees respectively by neighbor-joining. Bootstrap and jackknife values <50% support not

shown. The only strongly supported branch is the TSE-TSE clade. The HA-SC clade is moderately supported.

Maximum likelihood:

Figure F-45 shows the maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model HKY + G; base frequencies of A=0.2826, C=0.1812, G=0.2432, T=0.2930; the Ti/Tv ratio set to 1.3000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2005. The –ln L score is 1338.86140.

Figure F-46 shows the bootstrap consensus for maximum likelihood using the same Modeltest parameters. Bootstrap values with <50% support are not shown. Most branches collapse into a polytomy, however the Hepatozoon-Haemogregarina (HG) clade is well supported at 80. The other supported clade is the TSE-TSE monophyly at 90.

Figure F-47 shows the maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model GTR + G; base frequencies of A=0.2798, C=0.1834, G=0.2582, T=0.2786; substitution rate matrix of A-C=0.9345, A-G=2.3842, A-T=2.2262, C-G=0.1839, C-T=4.2324, G-T=1.0000; ASRV invariable sites = 0.5390 and variable sites with a gamma distribution of 0.8563. The –ln L score is 1338.86140.

Figure F-48 shows the bootstrap consensus of maximum likelihood using the same Modeltest parameters.

Dataset D:

Maximum parsimony:

Gaps as 5th base:

Figure F-49 shows single most parsimonious tree with gaps treated a 5th base and nucleotides weighted equally. The most parsimonious tree had a TL=786, CI=0.763, RI=0.582, and RC=0.582.

Figure F-50 shows the bootstrap and jackknife consensus tree by maximum parsimony with bootstrap and jackknife values <50% support not shown. Bootstrap values are represented above branches and jackknife values are represented below branches. Gaps were treated as 5th base and nucleotides weighted equally.

Gaps as missing data:

Figure F-51 shows the single most parsimonious tree with gaps treated a missing data and nucleotides weighted equally. *Trachemys scripta* was set as outgroup to the ingroup. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. The most parsimonious tree had a TL=707, CI=0.748, RI=0.571, and RC=0.427.

Figure F-52 shows the bootstrap and jackknife consensus tree by maximum parsimony with bootstrap and jackknife values <50% support not shown. Bootstrap values are represented above branches and jackknife values are represented below

branches. Gaps were treated as missing data and nucleotides were weighted equally. This tree shows high support for the *Kinosternon* monophyly and high support for the Old World-New World split as individual monophyletic units.

Neighbor joining:

Figures F-53 and F-54 show the cladogram and phylogram respectively with optimality criterion set to distance with a neighbor joining search and HKY85 distance correction algorithm. The ME value is 0.75164. In the phylogram, the number of substitutions per site is represented as the length of each branch.

Figure F-55 shows the bootstrap and jackknife consensus tree by neighbor-joining with bootstrap and jackknife values <50% support not shown. Bootstrap values are represented above branches and jackknife values are represented below branches. The ME value is 0.75164 for the trees. Similar to the maximum parsimony tree, these topologies show high support for the *Kinosternon* monophyly, and high support for the Old World-New World clades individually. The *Annamemys-Callagur* clade shows monophyly, however is weakly supported.

Figure F-56 shows the maximum likelihood cladogram using Modeltest parameters. The parameters included model GTR + G; base frequencies of A=0.37230, C=0.26210, G=0.12320, T=0.24240; substitution rate matrix of A-C=2.040100, A-G=7.027900, A-T=0.963000, C-G=0.483500, C-T=15.696500, G-T=1.0000; ASRV invariable sites = 0, and variable sites with a gamma distribution of 0.3167. *Trachemys*

scripta was set as outgroup to the ingroup and nucleotides were weighted equally. **Figure F-57** shows the maximum likelihood bootstrap consensus tree. Bootstrap values (>50%) are represented above each branch.

Figure 29 shows the absolute pairwise distances over four taxonomic levels including species, genera, family, and order. Each bar represents the maximum number of pairwise distances between each level. Distances within species (0 to 12, mean=5), genera (0 to 55, mean=14), family (20 to 33, mean=26), and order (33 to 48, mean=56) are based on the accepted taxonomy for Apicomplexa. Within *Caryospora bigenetica* the distance is 0 and within *Toxoplasma gondii* the distance is 1. Within *Cryptosporidum parvum,* the distance is 3 or less for all taxa except AF112576 which has a maximum of 6. Within *Haemogregarina* sp. the distance is 7 or less with the exception of MF5494 which has a distance of 12 or less.

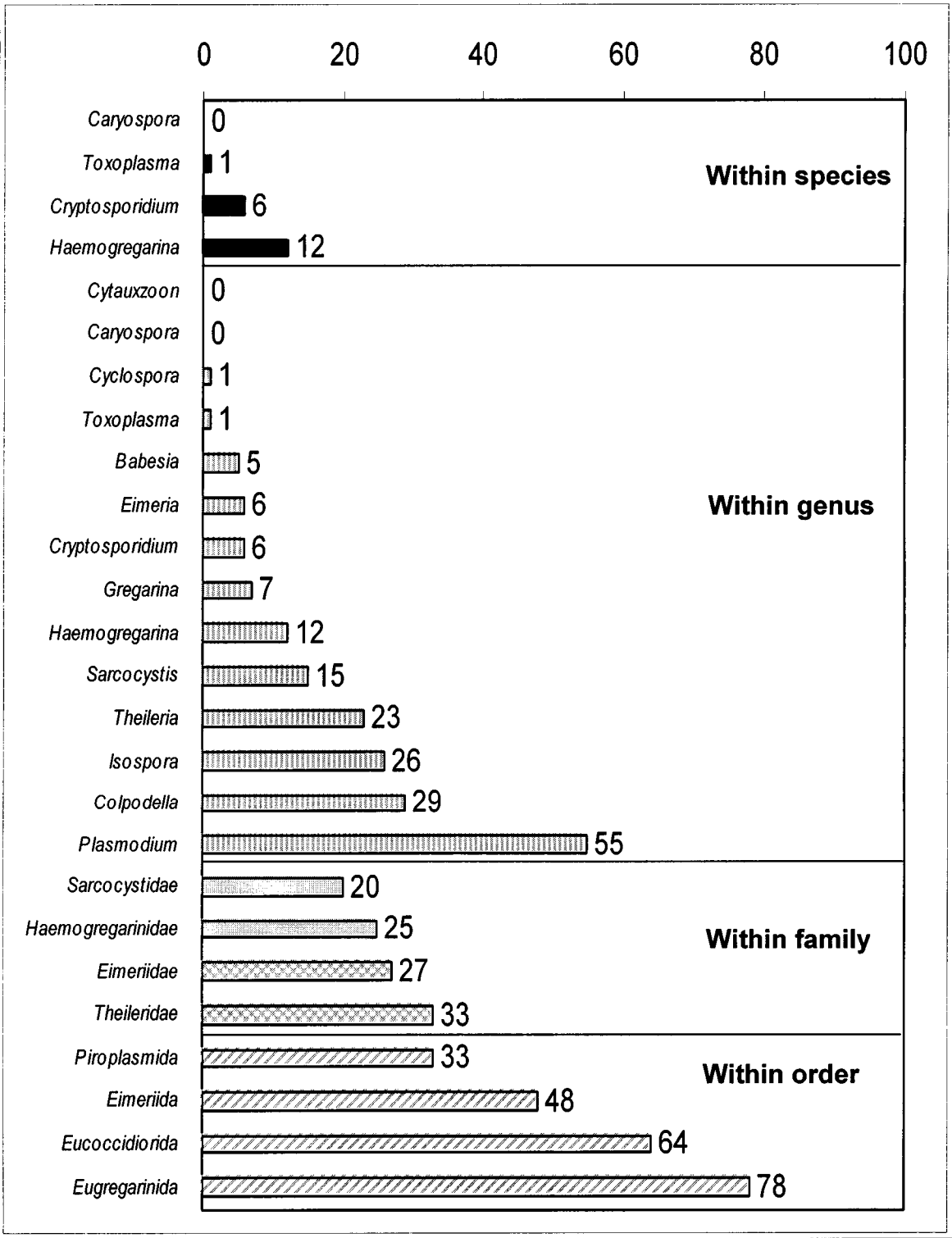
Taxa that consistently fell outside their accepted taxonomic rank for the following topologies included *Lankesterella minima* (Order Eucoccidiorida) often placed in the Order Eimeriida in Family Sarcocystidae or Family Eimeriidae. *Isospora suis* (Family Eimeriidae) was often placed in the Sarcocystidae. These particular taxa increased the absolute distances between taxa for each corresponding group.

Lankesterella minima increased the Eucoccidiorida from 41 to 64 by pairwise distance comparison to *Isospora suis*. When *Lankesterella* was placed within the Sarcyocystidae or Eimeriidae the distances dropped to 39 and 39 respectively. As in the

previous results, these higher numbers were due to comparison with *Isospora suis*. If *Isospora suis* is removed, however, the pairwise distances adjust with *Lankesterella* in Eimeriidae to 27 and to 43 with it in Sarcycocystidae. The removal of *Isospora suis* from the Eimeriidae decreased the pairwise distance does not affect the largest value. This taxon has the closest pairwise distance to *Hammondia*, *Neospora*, and *Toxplasma*, and therefore is most probably fits in the Sarcocystidae. *Isospora suis* has the smallest pairwise distance to *Hammonida* and *Hyaloklossia*.

Figure 30 shows the topologic hypotheses for examination of coevolution for parasite and turtle phylogenies. Cladograms A and B show coevolutionary speciation events for the parasite synchronous with speciation events of the host. Cladograms C and D show speciation events in turtles with similar parasites from the same locality. Cladograms C and D resembles the topologies for *Haemogregarina* and their turtle hosts.

Figure 29. Absolute pairwise distances over four taxonomic levels including species, genera, family, and order. Each bar represents the maximum number of pairwise distances between each level. Distances within species (0 to 12, mean=5), genera (0 to 55, mean=14), family (20 to 33, mean=26), and order (33 to 48, mean=56) are based on the accepted taxonomy for Apicomplexa.



DISCUSSION

This study provided the first exhaustive phylum-level phylogenetic reconstructions for the Apicomplexa for the sequences that were available. An additional nine apicomplexan genera were included that have not previously been evaluated (see Table 7). Understanding the taxonomy and phylogeny of these protozoans is essential for classification of parasite diversity, for future phylogenetic comparisons of other genes or organelles, in tracing epidemiology of protozoan diseases and in estimating divergence and evolutionary processes of Apicomplexa. By tracing the patterns of the evolutionary history of apicomplexans, the processes and adaptations can be illuminated. The application includes epidemiological investigation and parasitological control. This study also was the first to consider *Haemogregarina*, as well as several other taxa, for molecular phylogenetic reconstruction.

Based on the incomplete sequences available from GenBank for the alignment (see Figure 22 overview of alignment) and the impracticality in obtaining specimens and generating original sequence from all 70 taxa involved, different subsets of the 18S gene were evaluated based on the sequences that were available. This included an evaluation of the phylum Apicomplexa with the maximum number of characters for all taxa, a second subset for evaluation of the entire phylum that included many fewer characters but allowed *Haemogregarina* sequences to be included, and a third subset that focused exclusively on the family Haemogregarinidae to determine host specificity and coevolutionary status with the turtles. A partition homogeneity test as well as congruent

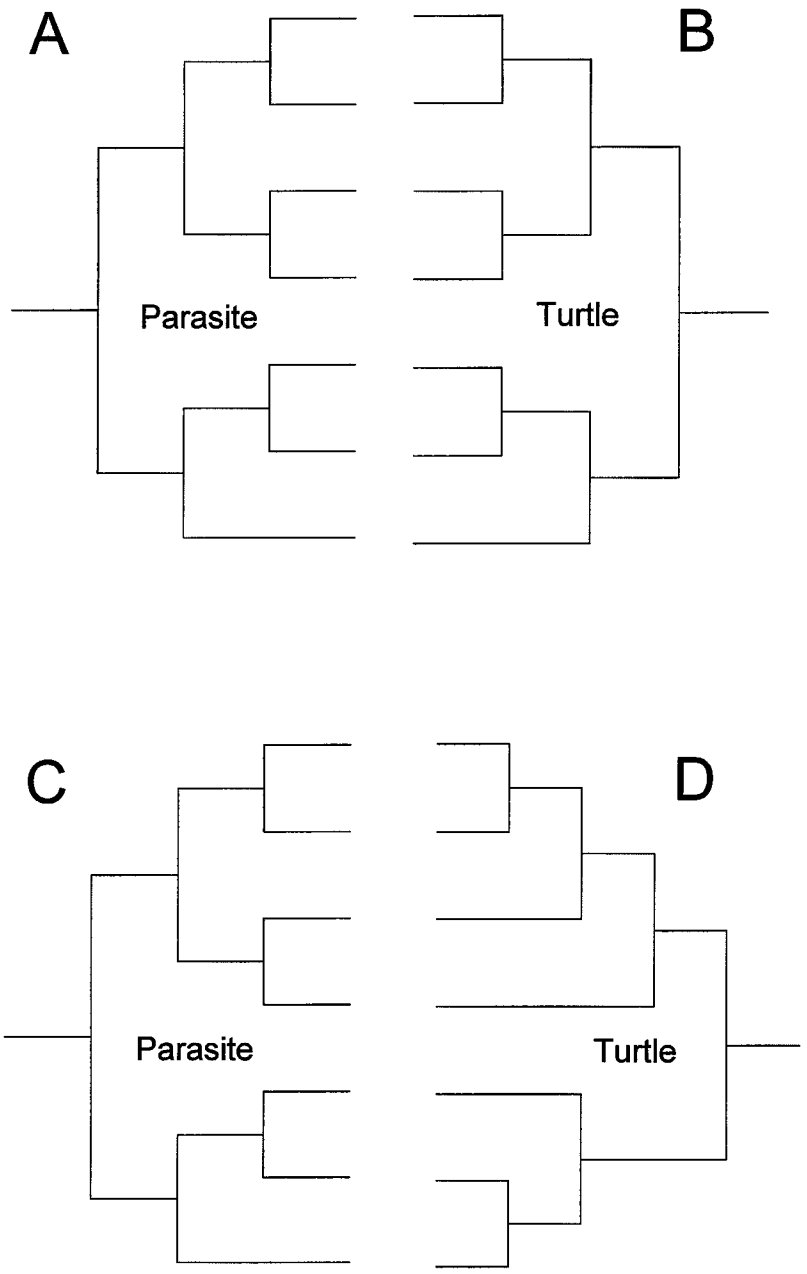


Figure 30. Topologic hypotheses for examination of coevolution for parasite and turtle phylogenies. Cladograms A and B show coevolutionary speciation events for the parasite synchronous with speciation events of the host. Cladograms C and D show speciation events in turtles with similar parasites from the same locality. Cladograms C and D resembles the topologies for *Haemogregarina* and their turtle hosts.

topologies for these datasets show that partitioning the 18S gene based on available sequence is appropriate for Apicomplexa.

Phylogenetic analyses of phylum Apicomplexa

The topologies produced from this investigation prove difficult to compare to previously published molecular phylogenetic analyses due to the significant differences in the number of genera included. Obornik et al. (2002) used apicoplast sequence and resolved the families Eimeriidae and Sarcocystidae within the Order Eimeriida in agreement with previously published data. The placement of all other taxa (*Babesia*, *Plasmodium*, and *Hepatozoon*) is questionable and appears to serve as a "catch all" clade for genera outside Eimeriidae and Sarcocystidae. Perkins & Keller (2001) present molecular data for haemogregarines, however do not give generic names and only classify as a "haemogregarine." It is not certain, therefore, the particular genera under study for that publication. Interestingly, Perkins & Keller show *Lankesterella* is grouped as sister taxa to *Eimeria*, which is the case for the current study. Due to the limited number of taxa in their study, comparisons beyond these taxa are difficult to explore. Morrison et al. (1997) provide evidence of monophyly of Sarcocystidae and monophyly of Piroplasmida, however further descriptions can not be concluded.

For all phylogenetic analyses in the current study, the taxonomic position of *Lankesterella* should be further examined. It is currently placed systematically within the

order Eucoccidiorida (with families Haemogregarinidae, Lankesterellidae, and Colpodellidae). For all current analyses, *Lankesterella* is phylogenetically most similar to the Eimeriidae. *Lankesterella minima* increased the Eucoccidiorida from 41 to 64 by pairwise distance comparison to *Colpodella*. When *Lankesterella* was placed within the Sarcyocystidae or Eimeriidae the distances dropped to 39 and 39 respectively. These higher numbers were due to comparison with *Isospora suis*. If *Isospora suis* is removed, however, the pairwise distances adjust with *Lankesterella* in Eimeriidae to 27 and to 33 with it in Sarcycocystidae. This suggests that the identification of *Isospora suis* (U97523) is misidentified in GenBank. The removal of *Isospora suis* from the Eimeriidae decreased the pairwise distance does not affect the largest value. *Isospora suis* has the smallest pairwise distance to *Hammonida* and *Hyaloklossia* and therefore is most probably fits in the Sarcocystidae. The *Isospora suis* isolate failed to group with its sister taxa *Isospora robini*, and therefore this isolate should be re-examined.

The phylogenetic trees performed with maximum likelihood optimality criterion are presented as Figures 23 (dataset A), and Figure 24 (dataset B). Figure 23 reveals a cladogram that represents the current NCBI systematic classifications with exception to *Lankesterella* and *Isospora suis* previously mentioned. The classification of the *Leidyana* monophyly with *Colpodella* and the monophyly of *Monocystis-Ophriocystis* within the Cryptosporidiidae is questioned. Figure 24 shows a cladogram that overall has less support than Figure 23. The Cryptosporidiidae is not monophyletic and most branches collapse as a polytomy.

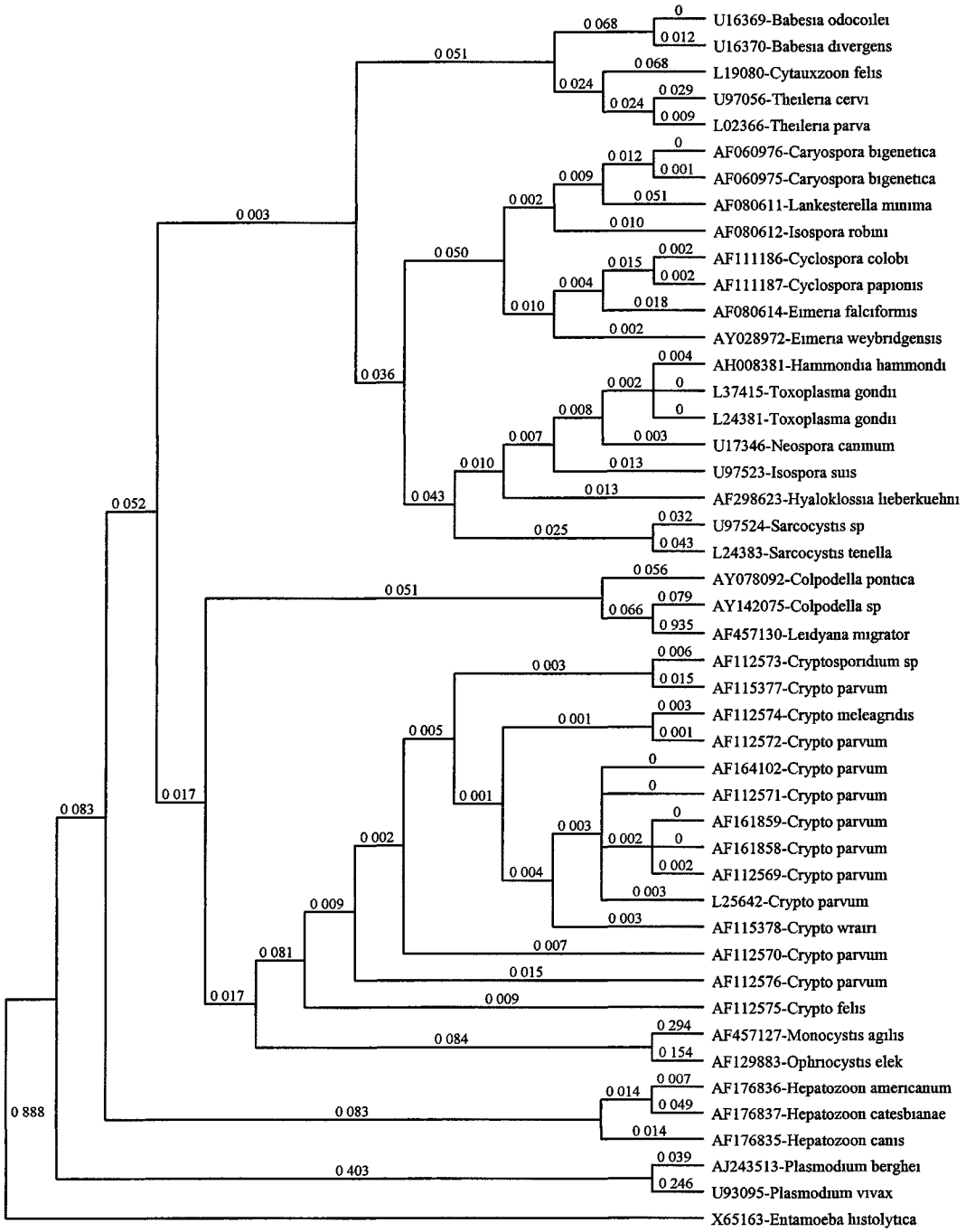


Figure 31. Dataset A: Maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model GTR + I + G; base frequencies of A=0.3265, C=0.1652, G=0.2313, T=0.2771; substitution rate matrix of A-C=1.2767, A-G=2.5466, A-T=1.8424, C-G=0.8904, C-T=4.8326, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.3531. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. The –ln L score is 9273.59391. This is the best supported tree for the Phyla-level analyses. The Cryptosporidiidae, Colpodellidae, Monocystidae, and Ophryocystidae form a monophyletic clade that disagrees systematically. *Leidyana* forms a monophyletic clade with *Colpodella* alternatively to its systematic order Eugregarinida. *Isospora suis* and *Lankesterella* continue to disagree with systematic position.

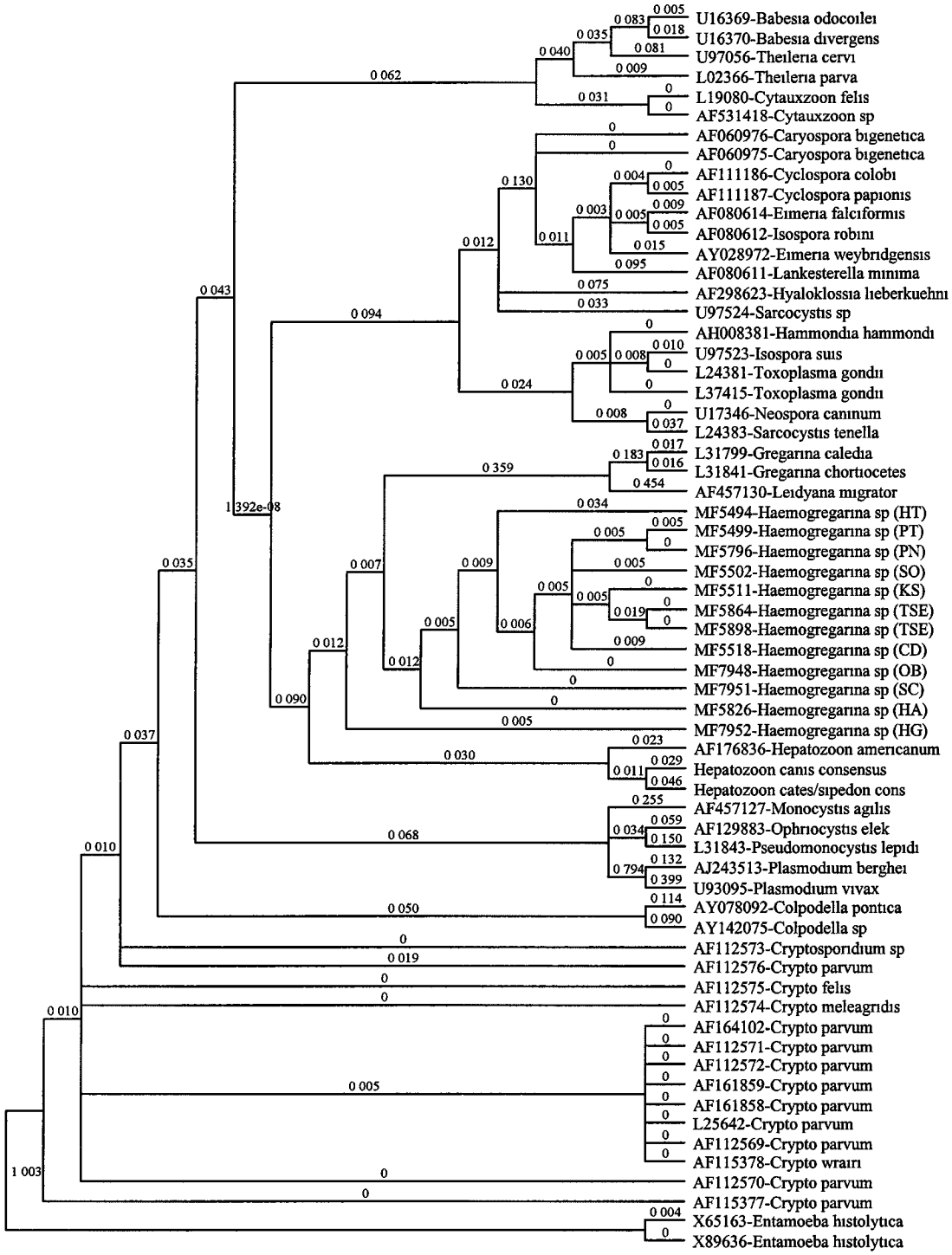


Figure 32. Dataset B: Maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model TrN + G; base frequencies of A=0.2937, C=0.1655, G=0.2484, T=0.2924; substitution rate matrix of A-C=1.6907, A-G=2.4927, A-T=1.5261, C-G=0.8853, C-T=5.1433, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2338. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally. With optimality criteria set to maximum likelihood, a heuristic search was performed with TBR branch swap method and ran until completion. The –ln L score is 3770.59511. This topology is almost identical to Figure 29 (different Modeltest parameters) yet differs in topology in the placement of the *Gregarina-Leidyana* monophyletic clade.

Based on the phylogenetic analyses for the phylum Apicomplexa, the placement of *Haemogregarina* was determined as monophyletic. These analyses show *Haemogregarina* as an individual genus and *Hepatozoon* as the closest sister group to *Haemogregarina.* The placement of *Haemogregarina* and *Hepatozoon* as a monophyletic unit support the status in the family Haemogregarinidae. It appears that *Monocystis* and *Ophriocystis* are the closest sister group taxa, and therefore these genera were used for outgroup comparison for the Haemogregarinidae phylogenetic analyses (dataset C).

Phylogenetic analyses of Family Haemogregarinidae

Based on the results from dataset C, it can be concluded that the parasites amplified for the current study are indeed *Haemogregarina* sp. (as identified morphologically in Chapter 2) and are a sister group to *Hepatozoon*. Based on analyses from datasets A and B, *Monocystis* and *Ophriocystis* were chosen as the closest sistergroup outside the Haemogregarinidae family. For maximum parsimony analyses, strict majority rule consenses collapse as polytomies most likely due to the vast similarities in the sequence. Distance analyses show short branches representative of evolutionary similarity between *Haemogregarina* isolates from turtles, however isolates from *Hepatozoon canis* and *Hepatozoon sipedon* show much greater substitutions per site. Maximum likelihood analyses reveal a distinct Old World-New World separation, however group *Hepatozoon* within *Haemogregarina* and show no generic distinction.

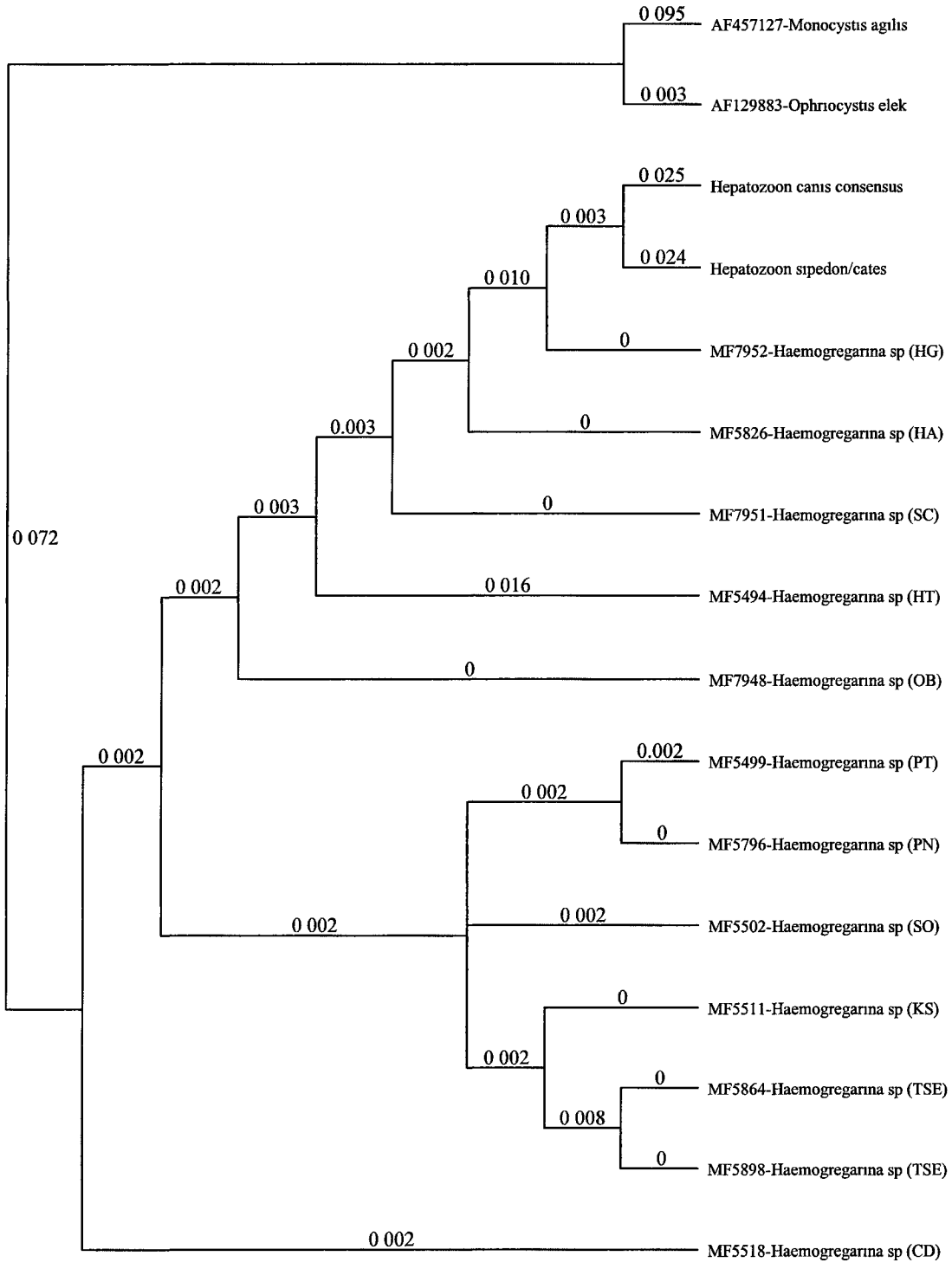


Figure 33. Dataset C: Maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model GTR + G; base frequencies of A=0.2798, C=0.1834, G=0.2582, T=0.2786; substitution rate matrix of A-C=0.9345, A-G=2.3842, A-T=2.2262, C-G=0.1839, C-T=4.2324, G-T=1.0000; ASRV invariable sites = 0.5390 and variable sites with a gamma distribution of 0.8563. *Monocystis* and *Ophriocystis* were set as monophyletic outgroup to the Apicomplexa and nucleotides were weighted equally. With optimality criteria set to maximum likelihood, a heuristic search was performed with TBR branch swap method and ran until completion. The –ln L score is 1338.86140.

Evaluation of *Haemogregarina* as individual species

When considering the phylogenetic species concept with respect to *Haemogregarina* sp. that are morphologically very similar to *Hepatozoon* sp., it is apparent that they are indistinguishable as distinct genera in some of the molecular phylogenetic analyses. *Haemogregarina* and *Hepatozoon* genera together form a monophyletic unit with respect to all Apicomplexa and are therefore in the same family or even congeneric. It appears that all *Haemogregarina* isolates with the exception of MF7952 are monophyletic. This sequence may indeed be a *Hepatozoon* isolate or even an additional unidentified genus. To clarify this, additional known *Hepatozoon* sequences will be required to enable resolution of this anomaly.

The within-group pairwise distances among all taxa from Haemogregarindae (*Haemogregarina* and *Hepatozoon* collectively) reach a maximum of 25 (Figure 29) which would suggest that the family Haemogregarinidae contains at least two genera. This is evident in the cladogram from Figure 32 (dataset B) which shows a monophyletic clade for *Hepatozoon* and a separate monophyletic clade for *Haemogregarina*. However this result is not without some ambiguity. In contrast, Figure 33 (dataset C) provides a paraphyletic *Haemogregarina* with respect to *Hepatozoon*. If this is correct, the traditional key distinguishing feature between these genera that *Haemogregarina* is transmitted by leech vectors may be an inaccurate classification element. However, this result may be attributed to outgroup sensitivity or differential datasets. It is also possible that using consensus *Hepatozoon* sequences for dataset C was erroneous. While based on the dataset C topologies, it is impossible to interpret *Haemogregarina* and *Hepatozoon* as

two distinct genera, the larger and more inclusive dataset B analyses support both genera. From the data analyzed here, it is not possible to definitively comment on the generic status of *Haemogregarina*.

It has been common practice to assign a new species name to the parasite when it is discovered in a new host taxa. This is unwarranted, however, since the host specificity remains uncertain (Ball, 1967; Chao & Ball, 1967; Siddall & Desser, 1991; Smith et al., 1996). In a comparison of Figure 29 in which the absolute pairwise distances are examined for Apicomplexans, the number of changes and evaluation of *Haemogregarina* species can be determined. For the *Haemogregarina* sp. in the *Pseudemys texana* and *Pseudemys nelsoni* monophyletic unit, the absolute pairwise distance is 1 suggesting that these are the same species. For the monophyletic *Haemogregarina* sp. in the *Trachemys scripta elegans* hosts (from different locations in Texas), the absolute pairwise distance is 0 suggesting that these are the same species. The pairwise distance of *Haemogregarina* sp. from *Pseudemys* sp. to *Trachemys* sp. is a distance of 5, and however is probably the same species. This is supported by the bifurcation of *Haemogregarina* in New World turtle hosts in Figure 33. The placement of *Haemogregarina* sp. MF7952 with *Hepatozoon* sp. (Figure 33) is not supported based on the absolute pairwise distance comparisons. The distances within *Haemogregarina* sp. is a maximum of 12, whereas the distances of *Haemogregarina* to *Hepatozoon* sp. has a distance range from 11 (MF7952) to 25 (MF5494). This suggests that *Haemogregarina* sp. from MF7952 should be classified as *Haemogregarina* sp. and not as *Hepatozoon* sp, however MF7952 does show the greatest evolutionary divergence among the *Haemogregarina* sp. taxa.

The pairwise distances between all species of *Haemogregarina* has a maximum value of 12 (see Figure 29) that occurs between *Haemogregarina* MF5494 and MF7952 and thus represents the extremes in evolutionary changes in the genus. Based on the pairwise distances for other apicomplexans (Figure 29), this value between these two genera suggests that MF5494 and MF7952 remain in the same genera (compared to *Plasmodium* at 55), however the distances are larger than for other *Haemogregarina* sp. and therefore probably different species. Figure 33 shows MF5518 outside the monophyletic clade including *Haemogregarina* and *Hepatozoon*. This may potentially represent another genera or species.

Host specificity and coevolution with turtle hosts

When considering host-specificity and terms of co-evolution, there are some interesting cases consider. For the Aquarena Springs locality, *Sternotherus* (*Kinosternon*) *odoratus*, *Trachemys scripta elegans*, *Pseudemys texana*, and *Pseudemys nelsoni* were collected. With the assumption that each turtle species has equal chance of leech attachment, it is also assumed that each turtle species has equal chance of parasite transmission through the leeches. It is interesting to note, however, that the monophyletic units for *Haemogregarina* were within *Pseudemys* hosts (*P. texana* and *P. nelsoni*) and separately within *Trachemys* hosts (*T. s. elegans* from Aquarena Springs and from Deanville, Texas locality). These monophyletic clades by turtle host genera, as opposed to monophyletic clades by host location, infer a host specificity for these particular *Haemogregarina* sp. The monophyly of *Haemogregarina* in *Sternotherus odoratus*

hosts (Aquarena Springs) with *Kinosternon sonorianse* hosts (captive from Alabama) also infers a familial lineage as opposed to a locality lineage. These results are preliminary, and additional sequences from additional taxa must be generated for elucidation of host-specificity. Manter's Rule (see Chapter 1) contends that if the same species, or two closely related species of hosts, exhibit a disjunct distribution and possess similar faunas, then the areas in which the hosts occur must have been contiguous in the past. It is evident from this rule that *Trachemys scripta elegans* from the disjunct localities shared a monophyletic ancestor.

Based on 20 years of studies at Aquarena Springs, there do not appear to be any unusual mortality or morbidity that may be attributed to *Haemogregarina* infection. Overall the turtles appeared to have constant low-levels of parasitemia (see Chapter 2). This suggests that the parasites are not particularly pathogenic. In cases where the parasitemia was high (e.g. 2%), this occurred in individuals that were stressed and potentially immunocompromised. Based on these phylogenetic analyses, it can be concluded that *Haemogregarina* is host specific, however does not appear to be pathogenic under normal conditions.

For maximum parsimony analyses for datasets A, B, and C, the treatment of gaps as a 5^{th} base and then as missing data provided slightly different results. When gaps were treated as a 5^{th} base, the topology had fewer polytomies and higher bootstrap and jackknife values. The overall topology did not differ significantly for a majority of the taxa. This may be attributed to a majority of the large gapped regions removal prior to all

phylogenetic analyses. In a comparison of the CI, RI, and RC values for maximum parsimony with gaps as a 5th base had higher scores for datasets C and D, whereas gaps as missing data had higher scores for datasets A and B (see Table 11). These values range from 0 for homoplastic characters to 1 for consistent characters. The higher scores reveal a reduction in the amount of phylogenetic noise. This noise is most notably in the loops where the majority of the gaps were inserted, whereas the stem regions remain highly conserved. The higher CI, RI, and RC indices for gaps as missing data for datasets A and B can be attributed to the number and placement of numerous gaps across the diverse dataset. Datasets C and D were much more similar across taxa, and therefore required few gaps to complete alignment. The treatment of gaps as a 5th base compared to treatment of gaps as missing data, can therefore be directly attributed to the diversity of taxa under study.

A partition homogeneity test was performed in PAUP* to determine if the two datasets A and B differed significantly. Under maximum parsimony optimality criteria and gaps treated as a 5th base, the p-value was not significant at 0.6704 which demonstrates equivalent partitions. With gaps treated as missing data with the partition homogeneity test, the p-value was 0.010 which demonstrates the partitions are not equivalent. This suggests that gaps treated as a 5th base is the most appropriate model for these particular datasets.

Summary from phylogenetic analyses

Phylogenetic analyses based on molecular data provided a reliable means to evaluate the phylogeny, taxonomy, host specificity, and coevolutionary status of *Haemogregarina* in naturally-occurring populations and translocated populations. It was determined that the previously published molecular phylogenies of Apicomplexa were incomplete in the number of taxa investigated and therefore difficult for comparison. However stable clades included the order Eimeriida with its families Eimeriidae and Sarcocystidae. The Eucoccidiorida does not appear to be monophyletic, nor does the Eugregarinida. The class Piroplasmida is monophyletic, however, the placement of *Cytauxzoon* remains questioned.

The placement of *Haemogregarina* does support a monophyletic unit, indicating a single genus for the parasites. The family-level evaluation of Haemogregarinidae does support a monophyletic clade, and shows *Hepatozoon* as sister group to *Haemogregarina*. Based on the phylogenetic analyses, it does appear that *Haemogregarina* is host specific, however this specificity does not appear to be pathogenic and remains in low levels of infection.

CHAPTER IV

IMPLICATIONS OF *HAEMOGREGARINA* INFECTION IN TURTLES

In light of increasing species extinction rates, primarily a consequence of human encroachment and habitat destruction, worldwide biodiversity is declining. Genetic homogeneity, especially in small populations, can lead to higher vulnerability to environmental factors and thus exacerbate the decline in biodiversity on local scales. Turtles are particularly susceptible to escalating extinction rates due to delayed sexual maturity, high egg and juvenile mortality, and long adult life-span with low natural mortality.

The Turtle Survival Alliance (TSA) is an IUCN (World Conservation Union) organization for the captive management of freshwater turtles and tortoises. The focus of the Turtle Survival Alliance lies in Asia where one-third of the world's turtle diversity is located, however virtually all of the Asian species are currently endangered or threatened. This organization was developed as a solution to the Asian Turtle Crisis which exploits turtles for human consumption, medicinal uses, turtle shell decorations and as pets. This conservation organization has recently established captive Assurance Colonies to maintain genetic diversity for future recovery of wild populations by maintaining them in captive populations. Assurance Colonies maintain threatened turtles which have been relocated from their troubled native habitat, illegally traded, and confiscated individuals. By sustaining these turtles in this worldwide conservation network, the long term goal is recovery efforts to place turtles back into wild populations. Turtles such as the Roti snake-necked turtle (*Chelodina*

mccordi) and the Painted terrapin (*Callagur borneoensis*) are listed by the TSA in the top 25 most endangered turtles for 2003. Several individuals of these two endangered species were translocated from Asia and are now part of a local Assurance Colony in Austin which were examined in the current study.

Translocations

The Asian Turtle Crisis has resulted in exhausted populations of turtles near China and now severely affects all species of turtles in Southeast Asia. The impact can be observed worldwide as North America, Africa, and Europe are shipping turtles to meet the high demand of turtles in Asia for consumption. In the past 10 years, the IUCN reported that the United States exported hundreds of thousands of softshell and snapping turtles and an additional 8 million red-eared sliders to China. The Turtle Survival Alliance has a global action plan to establish Assurance Colonies for reintroduction into natural habitats, and the United States has established a secure refuge for these endangered species until suitable habitats are available. The turtles included in these Assurance Colonies also protect turtles located in the United States such as the Bog Turtle (*Clemmys muhlenbergii*) and the Yellow-blotched map turtle (*Graptemys flavimaculata*).

Captive breeding programs which protect endangered turtles maintain biodiversity, but also has causes for concern. Translocation of these endangered species carries the risk of infectious disease exposure to naïve recipient populations, and recipient populations may in turn infect the translocated organisms. Captive husbandry can render these wild caught

turtles domesticated and unreturnable to wild populations if the opportunity were to arise. Captive management programs for reptiles often lack proper training including space requirements, proper cages, intensity of light, and feeding requirements. All of these factors may attribute to stress in the individual which can physiologically and immunogically render the individual at a disadvantage in the prevention of disease.

It is necessary to understand the efficacy of the translocation programs and the factors that ensure successful translocation survival. Successful programs should include natural habitats similar to the turtle's native environment, sufficient space to avoid overcrowding, egg-laying space, and adequate lighting and nutrition. These factors for success are not exclusive, however, and are multifactorial considering the individual's age, stress, infectious disease and potential of being immunocompromised. The recipient population's ecology, environment, carrying capacity, and competition for resources must also be considered. The emergence of disease most often occurs when the ecology of the host or pathogen is disrupted. Small populations are particularly impacted by disease since they are often in a close habitat range and lack substantial numbers to rebound from disease. Indoor and outdoor captive husbandry facilities must personalize the new environment to the individual needs of the turtle species.

Protocols for translocations

Several protocols delineate the screening, quarantine, and safeguards for translocated organisms, including fish, amphibians, mammals, and reptiles. Woodford (2000) outlines

quarantine and health screening protocols for wildlife prior to translocation and release into the wild that includes appropriate precautions applicable to transcontinental shipment of turtles. This article serves as a basis for evaluation and prevention of *Haemogregarina* parasite infections detected in Chapter 2 of the current study. These protocols include "a clinical evaluation of the health status of the source animals and those at the translocation destination, a period of quarantine, appropriate health screening procedures, a consideration of the legal and veterinary restrictions on translocation of wild animals to and from certain geographic areas and populations, and when necessary, pre-release treatment and immunization." These tenets will be discussed individually with respect to the worldwide turtle trade and the potential disease transmission within captive and wild populations of a particular coccidian *Haemogregarina* sp. that has been reported from all major continents.

1) Clinical evaluation of the health status

When Asian turtles are liberated from ill-fated meat-markets or illegal trade facilities, the primary objective is to protect the individual and relocate it to a secure location. This often involves packing the stressed, malnourished, and immunocompromised turtles into cardboard shipping boxes for several days or weeks en route to a captive husbandry facility. Upon arrival, there is usually brief visual inspection for apparent disease but inadequate health screening for potential infectious diseases. It is recommended that all translocated individuals undergo a thorough physical and histological examination for potential diseases that can be remedied. The cause of death of translocated turtles must also be fully investigated with a thorough necropsy involving blood analyses, major tissue dissection, and

examination for any possible diseases. Fortunately, both health screening and necropsies involve basic laboratory equipment and low costs. Physical examinations should be maintained for all organisms on a regular basis as preventative medicine. Blood smears and a PCR based assay should be performed to detect Apicomplexan haemoparasites such as *Haemogregarina*.

For wild indigenous populations, the evaluation of disease in turtles is more challenging. For example, Southwest Texas State University routinely examines turtles at Aquarena Springs for epiphytes, blood parasites (see Chapter 2), reproductive success, and maintains records involving growth of turtles, age and sex ratios for all turtles captured yearly. Most wild populations, however, lack this scrutiny and are sporadically sampled and rarely screened for health conditions. A wild population is certainly not invulnerable to established or introduced diseases, however probably lack the severity of infection that could accompany translocated turtles.

For all turtles examined in this study, blood smears were examined for the detection of blood parasites (see Chapter 2). Epiphytic parasites were also documented and physically or chemically (iodine) removed from turtles. In an effort to identify the parasites, both morphological comparisons (light microscopy) and molecular characterization (nucleotide sequence) were performed (see Chapter 2). The epidemiology of the *Haemogregarina* sp. was traced using phylogenetic trees to ascertain species divergence and host specificity. Based on these analyses, it was concluded that *Haemogregarina* sp. are indeed host-specific. The constant (Caskey, 1998 compared to current analyses 2003) low-levels of parasitemia

reveal that *Haemogregarina* does not appear to be pathogenic in the turtles for this study. This may be attributed to their non-pathogenicity in natural hosts. It is not certain, however, the level of parasitemia and pathogenicity to other non-native host genera. An introduced turtle that is not from this region, such as released from the pet trade or other anthropogenic influence, may cause severe immune responses.

2) a period of quarantine

For any infected individual, a period of quarantine is necessary to avoid transmission of infectious diseases. After a full health screening, the length of quarantine should be determined based on the disease encountered. If no disease discovered, a period of quarantine (ex. 90 days) with several health screenings periodically should be performed. It was discovered in the current study (see Chapter 2) that all captive populations had 0% parasitemia, and it was concluded that the removal of the leech vector was the explanation. In wild populations, however, removal of this leech vector and quarantine periods for *Haemogregarina* is not a reality. Periodic evaluation of these wild populations, however, is a realistic approach to ensuring *Haemogregarina* and any other disease remain at safe levels.

Evidence of congenital transmission has been described for the haemogregarine *Hepatozoon* in water snakes (Lowichik and Yaeger, 1987). This is another possible source of infection and provides an additional motivation for quarantine of infected individuals. Although evidence is lacking for haemoparasites, the apicomplexan coccidian *Eimeria* was evaluated for maternal transfer of antibodies via egg yolk in chickens (Smith et al., 1994).

This study showed that antibodies transferred to hatchlings provide a high immunity against a particular *Eimeria* species, and a partial immunity against a similar *Eimeria* species. In evaluation of congenital transmission of parasites as well as maternal transfer of antibodies, it is possible that maternal influence may play a role in the epidemiology of *Haemogregarina*. Further analyses are required for a conclusive understanding of this route of transmission.

3) leech control

It is also necessary during the period of quarantine to remove any external leeches that may harbor parasites and serve as potential vectors to other uninfected turtles. For the captive populations examined in the current study, the addition of copper sulfate has proved resourceful in removal of leeches. For the captive indoor populations, the addition of copper sulfate to the aquarium water was sufficient. For the captive outdoor population (Concordia Turtle Farm) there is the constant possibility of leeches entering the outdoor ponds by using predatory birds or other animals as dispersal mechanisms. Copper sulfate pentahydrate (EPA Registration Number 35896-19, purchasable from Agtrol Chemical Products) is registered for leech control in farm ponds and a concentration of 5 parts per million (ppm) is suggested for treatment. Multiple treatments may be required to eliminate leeches since encapsulated eggs are resistant to treatment. It appears (see Chapter 2) that proper leech control eliminates *Haemogregarina* infections in approximately less than three years. Therefore, removing the vector impedes the disease transmission, and the degradation of erythrocytes removes the parasites from peripheral blood detection. The parasites may remain undetected while residing in major organs, however, this would only be visible during necropsy.

For the current study, none of the individuals were quarantined for disease or leeches upon arrival at captive facilities. However, the translocated organisms from Asia to the uninfected captive recipient population should be further studied for other potentially pathogenic diseases. As an example, a *Haemogregarina*-infected turtle from Asia may harbor infected leeches that are not apparent upon visual inspection at arrival at the captive facility. This leech may attach to different turtles in the same aquarium and therefore infect a previously uninfected turtle resident of the captive population. Depending on the host specificity of this particular *Haemogregarina* species, this may potentially induce a pathogenic response in the new turtle host species. Another possible problem may include translocated Asian turtles that carry *Haemogregarina* parasites but lack the leech vector. If the leech vector is present at the captive facility, this may also transmit disease to an un-natural host.

4) Anti-parasitic drugs

Due to the considerable biochemical differences in coccidia and their hosts, anti-parasitic drugs are currently under evaluation that target metabolic mechanisms exclusive to the parasites. Apicomplexans have a plant-like plastid and shikimate pathway that is not present in the vertebrate hosts. The herbicide glyphosate has shown to reduce the infection of the coccidian *Toxoplasma* in mice by inhibiting this shikimate synthesis activity (Roberts et al., 1998). Research has shown that coccidia induce an immune response to produce antibodies (Graczyk & Cranfield, 1997), therefore immunizations against the parasites could

be another treatment option. Anti-parasitic drugs do not appear to be necessary in *Haemogregarina* infections since leech removal correlates with elimination of the infection. Anti-parasitic drugs would prove beneficial in the case that translocated turtles presented un-natural hosts to specific strains of *Haemogregarina*.

5) appropriate health screening procedures

Woodford (2000) outlines acceptable health screening protocols. This includes a physical exam with body weight and measurements. An examination by fecal flotation or smears can also reveal any intestinal coccidian parasites or helminths that can be treated orally. Blood smears and haematocrit can identify unusual blood cell counts, blood parasites such as *Haemogregarina*, and clinical disease reflected by an increase in leukocytes. Whole blood, serum or plasma ratios can be evaluated for other deficiencies or signs of infection. Rectal swabs and oral swabs can be cultured for bacterial or fungal infections that also can be treated with antibiotics and antifungals prior to reintroduction in a population. Ectoparasites can be removed and possibly prevented depending on the particular parasite involved.

Woodford's protocols (2000) are primarily specific to re-introduction of wild organisms to a new locality. It serves, however, as a standard for controlling the potentially devastating effects of wildlife disease and effective monitoring for turtle populations in both wild, semi-wild, and captive situations.

The current study was initiated to determine: a) presence of previously reported coccidian parasite *Haemogregarina* in endemic Texas and translocated Asian turtles; b) determination of the level of parasitemia in terms of defining pathogenicity, c) accurate identification of genera based on morphological and molecular characters; d) tracing the divergence and host-specificity based on phylogenetic analyses from molecular data, and e) elucidating the possible epidemiology and parasitological control for wild and captive populations. Due to the Asian turtle crisis and increasing threat of biodiversity loss in turtles, this parasite information was collected to gain a greater perspective in disease transmission for conservation purposes in turtles.

In the current study, an evaluation of wild, captive within the past year (wild caught, captive raised), and captive turtles were evaluated for *Haemogregarina* infections. It was determined (Chapter 1) that turtles in wild populations such as Aquarena Springs maintained a low-level parasitemia, whereas captive turtles lacked the parasite entirely. This was attributed to the removal of the intermediate vector leech by copper sulfate addition to the water. The parasites do not appear to be pathogenic in these natural hosts, however experimental transfer studies have shown severe pathogenic responses in unnatural hosts (Wozniak et al., 1996) It was concluded in the current study (Chapter 2) that the parasites appear to be host-specific but do not appear to be pathogenic. When comparing the identification of *Haemogregarina* sp. methods of light microscopy to molecular techniques, it was determined that both techniques were useful in an accurate evaluation of the epidemiology (Chapter 2). In phylogenetic reconstruction of *Haemogregarina* using molecular characters, it was determined that there was host-specificity for a particular genera

of turtles as opposed to a lack of host-specificity from a generalized environment. The host-specificity was apparent by the monophyletic clades for a specific turtle genera and a lack of monophyly for turtle hosts from the same locality (Chapter 3).

For wild populations, there are additional implications with respect to disease investigation and conservation such as accessibility to all individuals for clinical evaluation, immigrant species as potential disease carriers, and inability to control leech vectors. Exotic introductions and *in situ* conservation projects (semi-wild populations) risk exposure to epidemiological agents. Within captive managed populations, assurance conservation colonies and commercial farming colonies may be a source of disease removal. As seen in the current *Haemogregarina* study, it appears that captive management and thus control of leech vector exposure, rendered the parasite undetectable and possibly eliminated in captive turtles held more than 3 years. Since this was the first examination of *Haemogregarina* parasitemia in these turtles, it is not known if the turtles in captivity for 3 years carried this infection or not. Since these turtles were also translocated from Asia, it assumed that they carried the same parasites as the turtles translocated in the past year. The period in captivity is expected to eliminate *Haemogregarina* infection due to the removal of the leech vector. The export of more than one million red eared sliders (*Trachemys scripta elegans*) yearly from captive localities such as the one studied in Louisiana to overseas are potential disease-carriers to recipient populations such as intestinal coccidia, trypanosomes, or bacterial infections. These exported *Trachemys* are not vectors with respect to *Haemogregarina* sp. since all turtles from this location were negative for parasitemia.

The multifactorial influence of population and evolutionary ecology regarding host-parasite relationships remains an intricate situation that requires further investigation. By understanding the transmission, pathogenicity, infection, and life history of these parasites, a better understanding of control methods for infection can be derived. With the worldwide biodiversity crisis affecting turtles, any effort to prevent the impact is significant. The future of the existence of turtles is in part dependent on Assurance Colonies to prevent extinction of endangered species. Certain guidelines are essential, however, to ensure these colonies are successful and prevent inadvertent deleterious effectors.

APPENDIX A

TISSUE COLLECTION AND TURTLE VOUCHERS

All blood samples were collected by Gina Lobban and stored in blood storage buffer at -80C as part of the Forstner permanent tissue collection.

	A	B	C	D	E	F	G	H	I	J	K	L
1	MF #	Collector #	Family	Genus	Species	Subspecies	Sex	Specific Locality	County	State	Country	Date Collected
2	MF# 5473	GLL #116	Batagurıdae	Callagur	borneoensıs		F	Waterlife	Hays	TX	USA	02/09/02
3	MF# 5474	GLL #117	Batagurıdae	Callagur	borneoensıs		F	Waterlife	Hays	TX	USA	02/09/02
4	MF# 5475	GLL #118	Batagurıdae	Callagur	borneoensıs		F	Waterlife	Hays	TX	USA	02/09/02
5	MF# 5476	GLL #119	Batagurıdae	Callagur	borneoensıs		F	Waterlife	Hays	TX	USA	02/09/02
6	MF# 5477	GLL #120	Batagurıdae	Callagur	borneoensıs		F	Waterlife	Hays	TX	USA	02/09/02
7	MF# 5478	GLL #121	Batagurıdae	Callagur	borneoensıs		F	Waterlife	Hays	TX	USA	02/09/02
8	MF# 5479	GLL #122	Batagurıdae	Callagur	borneoensıs		F	Waterlife	Hays	TX	USA	02/09/02
9	MF# 5472	GLL #125	Chelıdae	Chelodına	mccordı		F	Waterlife	Hays	TX	USA	02/09/02
10	MF# 5483	GLL # 127	Chelıdae	Chelodına	sıebenrockı		F	Waterlife	Hays	TX	USA	02/09/02
11	MF# 5482	GLL # 126	Chelıdae	Chelodına	sıebenrockı		F	Waterlife	Hays	TX	USA	02/09/02
12	MF# 5836	GLL #357	Chelıdae	Chelodına	sıebenrockı		M	Waterlife	Hays	TX	USA	04/06/02
13	MF# 5837	GLL #358	Chelıdae	Chelodına	sıebenrockı		M	Waterlife	Hays	TX	USA	04/06/02
14	MF# 5838	GLL #359	Chelıdae	Chelodına	sıebenrockı		M	Waterlife	Hays	TX	USA	04/06/02
15	MF# 5839	GLL #360	Chelıdae	Chelodına	sıebenrockı		F	Waterlife	Hays	TX	USA	04/06/02
16	MF# 5481	GLL #124	Chelıdae	Chelodına	mccordı		F	Waterlife	Hays	TX	USA	02/09/02
17	MF# 5569	GLL # 213	Emydınae	Chrysemys	pıcta	bellı	M	Concordıa Turtle Farm, Wildsville, LA		LA	USA	03/15/02
18	MF# 5518	GLL # 162	Batagurıdae	Cyclemys	dentata		M	Guthrıe turtle farms		AL	USA	03/14/02
19	MF# 5525	GLL # 169	Batagurıdae	Cyclemys	dentata		M	Guthrıe turtle farms		AL	USA	03/14/02
20	MF# 5526	GLL # 170	Batagurıdae	Cyclemys	dentata		M	Guthrıe turtle farms		AL	USA	03/14/02
21	MF# 5512	GLL # 156	Batagurıdae	Cyclemys	dentata		F	Guthrıe turtle farms		AL	USA	03/14/02
22	MF# 5840	GLL #361	Chelıdae	Elseya	branderhorstıı		F	Waterlife	Hays	TX	USA	04/06/02
23	MF# 5841	GLL #362	Chelıdae	Elseya	branderhorstıı		M	Waterlife	Hays	TX	USA	04/06/02
24	MF# 5842	GLL #363	Chelıdae	Elseya	branderhorstıı		M	Waterlife	Hays	TX	USA	04/06/02
25	MF# 5843	GLL #364	Chelıdae	Elseya	branderhorstıı		F	Waterlife	Hays	TX	USA	04/06/02
26	MF# 5844	GLL #365	Chelıdae	Elseya	branderhorstıı		F	Waterlife	Hays	TX	USA	04/06/02
27	MF# 5845	GLL #366	Chelıdae	Elseya	branderhorstıı		F	Waterlife	Hays	TX	USA	04/06/02
28	MF# 5846	GLL #367	Chelıdae	Elseya	branderhorstıı		M	Waterlife	Hays	TX	USA	04/06/02
29	MF# 5847	GLL #368	Chelıdae	Elseya	branderhorstıı		F	Waterlife	Hays	TX	USA	04/06/02
30	MF# 5848	GLL #369	Chelıdae	Elseya	branderhorstıı		M	Waterlife	Hays	TX	USA	04/06/02
31	MF# 5849	GLL #370	Chelıdae	Elseya	branderhorstıı		F	Waterlife	Hays	TX	USA	04/06/02
32	MF# 5850	GLL #371	Chelıdae	Elseya	branderhorstıı		F	Waterlife	Hays	TX	USA	04/06/02
33	MF# 5489	GLL # 133	Chelıdae	Emydura	subglobosa		F	Waterlife	Hays	TX	USA	02/09/02
34	MF# 5490	GLL # 134	Chelıdae	Emydura	subglobosa		F	Waterlife	Hays	TX	USA	02/09/02
35	MF# 5491	GLL # 135	Chelıdae	Emydura	subglobosa		M	Waterlife	Hays	TX	USA	02/09/02
36	MF# 5492	GLL # 136	Chelıdae	Emydura	subglobosa		F	Waterlife	Hays	TX	USA	02/09/02
37	MF# 5471	GLL #114	Chelıdae	Emydura	subglobosa		F	Waterlife	Hays	TX	USA	02/09/02
38	MF# 5480	GLL #123	Batagurıdae	Geoemyda	nıgrıcan		F	Waterlife	Hays	TX	USA	02/09/02
39	MF# 5885	GLL #406	Emydınae	Graptemys	versa		M	Capıtol Aggregate, Marble Falls		TX	USA	05/01/02
40	MF# 5886	GLL #407	Emydınae	Graptemys	versa		M	Capıtol Aggregate, Marble Falls		TX	USA	05/01/02
41	MF# 5887	GLL #408	Emydınae	Graptemys	versa		M	Capıtol Aggregate, Marble Falls		TX	USA	05/01/02
42	MF# 5888	GLL #409	Emydınae	Graptemys	versa		M	Capıtol Aggregate, Marble Falls		TX	USA	05/01/02
43	MF# 5488	GLL # 132	Emydınae	Graptemys	barberi		M	Waterlife	Hays	TX	USA	02/09/02
44	MF# 5493	GLL # 137	Batagurıdae	Hardella	thurjıı		F	Waterlife	Hays	TX	USA	02/09/02
45	MF# 5494	GLL # 138	Batagurıdae	Hardella	thurjıı		M	Waterlife	Hays	TX	USA	02/09/02
46	MF# 5811	GLL #332	Batagurıdae	Heosemys	grandıs		U	Waterlife	Hays	TX	USA	04/06/02
47	MF# 5812	GLL #333	Batagurıdae	Heosemys	grandıs		F	Waterlife	Hays	TX	USA	04/06/02
48	MF# 5813	GLL #334	Batagurıdae	Heosemys	grandıs		F	Waterlife	Hays	TX	USA	04/06/02
49	MF# 5814	GLL #335	Batagurıdae	Heosemys	grandıs		F	Waterlife	Hays	TX	USA	04/06/02
50	MF# 5817	GLL #338	Batagurıdae	Heosemys	grandıs		U	Waterlife	Hays	TX	USA	04/06/02
51	MF# 5818	GLL #339	Batagurıdae	Heosemys	grandıs		M	Waterlife	Hays	TX	USA	04/06/02
52	MF# 5819	GLL #340	Batagurıdae	Heosemys	grandıs		F	Waterlife	Hays	TX	USA	04/06/02
53	MF# 5820	GLL #341	Batagurıdae	Heosemys	grandıs		F	Waterlife	Hays	TX	USA	04/06/02
54	MF# 5821	GLL #342	Batagurıdae	Heosemys	grandıs		F	Waterlife	Hays	TX	USA	04/06/02
55	MF# 5822	GLL #343	Batagurıdae	Heosemys	grandıs		M	Waterlife	Hays	TX	USA	04/06/02
56	MF# 5823	GLL #344	Batagurıdae	Heosemys	grandıs		F	Waterlife	Hays	TX	USA	04/06/02
57	MF# 5828	GLL #349	Batagurıdae	Heosemys	grandıs		F	Waterlife	Hays	TX	USA	04/06/02
58	MF# 5829	GLL #350	Batagurıdae	Heosemys	grandıs		F	Waterlife	Hays	TX	USA	04/06/02
59	MF# 5830	GLL #351	Batagurıdae	Heosemys	grandıs		F	Waterlife	Hays	TX	USA	04/06/02
60	MF# 5831	GLL #352	Batagurıdae	Heosemys	grandıs		U	Waterlife	Hays	TX	USA	04/06/02

	A	M	N	O	P	Q	R	S	T	U	V
1	MF #	Additional Data	PlastronLength/SVL	PlastronWidth/TL	CarapaceLength	CarapaceWidth	Wgt	W/C/WCCR	% parasitemia	parasite mmts (microns)	PCR test
2	MF# 5473	pit # 036-825-863, captive born 1999	n/a	n/a	n/a	n/a	4 5 lbs	C	0	n/a	neg
3	MF# 5474	pit # 036-822-103, captive born, 4 yrs , captive born 1999	n/a	n/a	n/a	n/a	16 27 oz	C	0	n/a	
4	MF# 5475	pit # 037-090-035, captive born, 4 yrs , captive born 1999	n/a	n/a	n/a	n/a	14 16 oz	C	0	n/a	
5	MF# 5476	pit # 036-839-781, captive born, 4 yrs , captive born 1999	n/a	n/a	n/a	n/a	11 98 oz	C	0	n/a	neg
6	MF# 5477	pit # 037-092-373, captive born, 4 yrs , captive born 1999	n/a	n/a	n/a	n/a	15 77 oz	C	0	n/a	
7	MF# 5478	pit # 036-626-879, captive born, 4 yrs , captive born 1999	n/a	n/a	n/a	n/a	13 23 oz	C	0	n/a	
8	MF# 5479	pit # 036-608-858, captive born, 4 yrs , captive born 1999	n/a	n/a	n/a	n/a	16 34 oz	C	0	n/a	neg
9	MF# 5472	pit # 122-567-491A, wild born, 5 yr captive	n/a	n/a	n/a	n/a	891 g	WCCR	0	n/a	neg
10	MF# 5483	captive born 1998	n/a	n/a	n/a	n/a	279 g	C	0	n/a	
11	MF# 5482	captive born 1998	n/a	n/a	n/a	n/a	357 g	WCCR	0	n/a	neg
12	MF# 5836	pit#122-529-322A, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	1460 g	WCCR	0	n/a	neg
13	MF# 5837	pit#122-565-663A, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	1940 g	WCCR	0	n/a	
14	MF# 5838	pit#122-937-130A, wild born, 5 yr, captive	n/a	n/a	n/a	n/a	1040 g	WCCR	0	n/a	neg
15	MF# 5839	pit#122-526-293A, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	3380 g	WCCR	0	n/a	
16	MF# 5481	pit # 122-555-652A, wild born, 5 yr captive	n/a	n/a	n/a	n/a	891 g	WCCR	0	n/a	
17	MF# 5569	no markings, dense population	108 mm	73 mm	n/a	n/a	200 g	C	0	n/a	neg
18	MF# 5518	wild born, 3 yr Captive	148 mm	103 mm	n/a	n/a	520 g	WCCR	0 003	n/a	pos
19	MF# 5525	wild born, 3 yr Captive	156 mm	111 mm	n/a	n/a	640 g	WCCR	0	n/a	
20	MF# 5526	wild born, 3 yr Captive	129 mm	97 mm	n/a	n/a	400 g	WCCR	no slides	n/a	
21	MF# 5512	wild born, 3 yr Captive	154 mm	115 mm	n/a	n/a	640 g	WCCR	0	n/a	neg
22	MF# 5840	pit#036-833-331, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	1800 g	WCCR	0	n/a	neg
23	MF# 5841	pit#036-791-372, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	1180 g	WCCR	0	n/a	
24	MF# 5842	pit#036-788-520, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	480 g	WCCR	0	n/a	neg
25	MF# 5843	pit#122-524-455A, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	2000 g	WCCR	0	n/a	
26	MF# 5844	pit#122-928-711A wild born, 5 yr Captive	n/a	n/a	n/a	n/a	1900 g	WCCR	0	n/a	neg
27	MF# 5845	pit#036-599-352, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	n/a	WCCR	0	n/a	
28	MF# 5846	pit#036-588-818, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	1020 g	WCCR	0	n/a	neg
29	MF# 5847	pit#122-472-651A, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	1960 g	WCCR	0	n/a	
30	MF# 5848	pit#036-620-820, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	760 g	WCCR	0	n/a	neg
31	MF# 5849	pit#036-805-883, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	1260 g	WCCR	0	n/a	
32	MF# 5850	pit#122-926-465A, wild born, 5 yr Captive	n/a	n/a	n/a	n/a	1500 g	WCCR	0	n/a	neg
33	MF# 5489	captive born, 2 yrs	n/a	n/a	n/a	n/a	340 g	C	0	n/a	neg
34	MF# 5490	captive born, 2 yrs	n/a	n/a	n/a	n/a	215 g	C	0	n/a	
35	MF# 5491	captive born, 2 yrs	n/a	n/a	n/a	n/a	286 g	C	0	n/a	neg
36	MF# 5492	captive born, 2 yrs	n/a	n/a	n/a	n/a	293 g	C	0	n/a	
37	MF# 5471	pit # 036-590-037, wild caught, 7 yr captive	n/a	n/a	n/a	n/a	2 08 kg	WCCR	0	n/a	neg
38	MF# 5480	pit # 122-524-764A	n/a	n/a	n/a	n/a	537 g	WCCR	0	n/a	neg
39	MF# 5885		158 mm	114 mm	197 mm	149 mm	1 75 lbs	W	0	n/a	neg
40	MF# 5886		93 mm	63 mm	111 mm	79 mm	0 5 lbs	W	0 01	6 x 2	
41	MF# 5887		79 mm	52 mm	97 mm	68 mm	0 25 lbs	W	0 08	14 x 4	
42	MF# 5888		81 mm	56 mm	99 mm	74 mm	0 25 lbs	W	0 51	4-13 x 4-5	pos
43	MF# 5488	captive born, 8 yrs	n/a	n/a	n/a	n/a	235 g	C	0	n/a	neg
44	MF# 5493	wild born, 3 yr Captive	n/a	n/a	n/a	n/a	352 g	WCCR	0	n/a	neg
45	MF# 5494		n/a	n/a	n/a	n/a	453 g	WCCR	0 07	10 x 6	pos
46	MF# 5811	HG141, 3 mo Captive, TSA seizure (from Malaysia/Indonesia)	n/a	n/a	n/a	n/a	7500 g	W	0 11	13 x 4	
47	MF# 5812	HG52, 3 mo Captive, TSA seizure (from Malaysia/Indonesia)	n/a	n/a	n/a	n/a	2520 g	W	0 12	14 x 6	
48	MF# 5813	HG108, 3 mo Captive, TSA seizure (from Malaysia/Indonesia)	n/a	n/a	n/a	n/a	1680 g	W	0 68	12 x 5	
49	MF# 5814	HG113?, 3 mo Cative, TSA seizure (from Malaysia/Indonesia)	n/a	n/a	n/a	n/a	2900 g	W	0 17	20 x 8	pos
50	MF# 5817	HG64, 3 month captive	n/a	n/a	n/a	n/a	4660 g	W	0 01	3-8 x 4-7	neg
51	MF# 5818	HG90 or 97, 3 month captive	n/a	n/a	n/a	n/a	3860 g	W	0 22	14-15 x 4-6	
52	MF# 5819	HG2101 3 mo Captive	n/a	n/a	n/a	n/a	3100 g	W	0 13	16 x 5	
53	MF# 5820	HG52?, 3 mo Captive, TSA seizure	n/a	n/a	n/a	n/a	3620 g	W	1 14	13 x 4	pos
54	MF# 5821	HG61, 3 mo Captive	n/a	n/a	n/a	n/a	3580 g	W	0 52	13 x 5	
55	MF# 5822	HG 96, 3 mo Captive, TSA seizure	n/a	n/a	n/a	n/a	3440 g	W	0 56	14 x 6	
56	MF# 5823	HG225, 3 mo Captive, TSA seizure	n/a	n/a	n/a	n/a	3780 g	W	0 15	12 5 x 6	pos
57	MF# 5828	HG161, 3 mo Cpative	n/a	n/a	n/a	n/a	4040 g	W	0 1	10 x 3	pos
58	MF# 5829	Hg79, 3 mo Captive	n/a	n/a	n/a	n/a	3540 g	W	2 1	12-18 x 5-9	pos
59	MF# 5830	HG102, 3 mo Captive	n/a	n/a	n/a	n/a	2400 g	W	0 8	15 x 6	
60	MF# 5831	HG65, 3 mo Captive	n/a	n/a	n/a	n/a	2520 g	W	0 08	14 x 5	pos

	A	B	C	D	E	F	G	H	I	J	K	L
1	MF #	Collector #	Family	Genus	Species	Subspecies	Sex	Specific Locality	County	State	Country	Date Collected
61	MF# 5832	GLL #353	Batagundae	Heosemys	grandis		F	Waterlife	Hays	TX	USA	04/06/02
62	MF# 5833	GLL #354	Batagundae	Heosemys	grandis		U	Waterlife	Hays	TX	USA	04/06/02
63	MF# 5834	GLL #355	Batagundae	Heosemys	grandis		U	Waterlife	Hays	TX	USA	04/06/02
64	MF# 5835	GLL #356	Batagundae	Heosemys	grandis		F	Waterlife	Hays	TX	USA	04/06/02
65	MF#7952	GLL#440	Batagundae	Heosemys	grandis		M	Waterlife	Hays	TX	USA	
66	MF# 5826	GLL #347	Batagundae	Hieremys	annandalii		M	Waterlife	Hays	TX	USA	04/06/02
67	MF# 5827	GLL #348	Batagundae	Hieremys	annandalii		F	Waterlife	Hays	TX	USA	04/06/02
68	MF# 5511	GLL # 155	Kinosternidae	Kinosternon	sonoriense		M	Guthrie turtle farms		AL	USA	03/14/02
69	MF# 5513	GLL # 157	Kinosternidae	Kinosternon	sonoriense		M	Guthrie turtle farms		AL	USA	03/14/02
70	MF# 5514	GLL # 158	Kinosternidae	Kinosternon	sonoriense		F	Guthrie turtle farms		AL	USA	03/14/02
71	MF# 5484	GLL # 128	Emydinae	Malaclemys	terrapin		F	Waterlife	Hays	TX	USA	02/09/02
72	MF# 5485	GLL # 129	Emydinae	Malaclemys	terrapin		F	Waterlife	Hays	TX	USA	02/09/02
73	MF#5486	GLL # 130	Emydinae	Malaclemys	terrapin		M	Waterlife	Hays	TX	USA	02/09/02
74	MF# 5487	GLL # 131	Emydinae	Malaclemys	terrapin		M	Waterlife	Hays	TX	USA	02/09/02
75	MF# 5456	GLL #3	Batagundae	Orlitia	borneensis			Waterlife	Hays	TX	USA	01/18/02
76	MF# 5457	GLL #4	Batagundae	Orlitia	borneensis			Waterlife	Hays	TX	USA	01/18/02
77	MF#5454	GLL #1	Batagundae	Orlitia	borneensis			Waterlife	Hays	TX	USA	01/18/02
78	MF# 5455	GLL #2	Batagundae	Orlitia	borneensis			Waterlife	Hays	TX	USA	01/18/02
79	MF# 5815	GLL #336	Batagundae	Orlitia	borneensis		U	Waterlife	Hays	TX	USA	04/06/02
80	MF# 5816	GLL #337	Batagundae	Orlitia	borneensis		F	Waterlife	Hays	TX	USA	04/06/02
81	MF# 5824	GLL #345	Batagundae	Orlitia	borneensis		U	Waterlife	Hays	TX	USA	04/06/02
82	MF#7947	GLL#435	Batagundae	Orlitia	borneensis			Waterlife	Hays	TX	USA	
83	MF# 7948	GLL #346	Batagundae	Orlitia	borneensis		F	Waterlife	Hays	TX	USA	04/06/02
84	MF#7949	GLL#437	Batagundae	Orlitia	borneensis			Waterlife	Hays	TX	USA	
85	MF# 5507	GLL # 151	Pelomedusidae	Pelusios	subniger		M	Guthrie turtle farms		AL	USA	03/14/02
86	MF# 5516	GLL # 160	Pelomedusidae	Pelusios	subniger		M	Guthrie turtle farms		AL	USA	03/14/02
87	MF# 5519	GLL # 163	Pelomedusidae	Pelusios	subniger		F	Guthrie turtle farms		AL	USA	03/14/02
88	MF# 5517	GLL # 161	Chelidae	Phrynops	gibbus		F	Guthrie turtle farms		AL	USA	03/14/02
89	MF# 5520	GLL # 164	Chelidae	Phrynops	gibbus		F	Guthrie turtle farms		AL	USA	03/14/02
90	MF# 5524	GLL # 168	Chelidae	Phrynops	gibbus		M	Guthrie turtle farms		AL	USA	03/14/02
91	MF# 5853	GLL #374	Chelidae	Phrynops	geoffroanus		F	Waterlife	Hays	TX	USA	04/06/02
92	MF# 5854	GLL #375	Chelidae	Phrynops	geoffroanus		M	Waterlife	Hays	TX	USA	04/06/02
93	MF# 5855	GLL #376	Chelidae	Phrynops	geoffroanus		M	Waterlife	Hays	TX	USA	04/06/02
94	MF# 5530	GLL #175	Chelidae	Platemys	platycephala		F	Guthrie turtle farms		AL	USA	03/14/02
95	MF# 5531	GLL # 174	Chelidae	Platemys	platycephala		M	Guthrie turtle farms		AL	USA	03/14/02
96	MF# 5796	GLL #317	Emydinae	Pseudemys	nelsoni		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
97	MF# 5799	GLL #320	Emydinae	Pseudemys	nelsoni		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
98	MF# 5459	GLL #14	Emydinae	Pseudemys	texana		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
99	MF# 5460	GLL #15	Emydinae	Pseudemys	texana		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
100	MF# 5461	GLL #16	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
101	MF# 5462	GLL #17	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
102	MF# 5463	GLL #18	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
103	MF# 5464	GLL #19	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
104	MF# 5465	GLL #20	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
105	MF# 5466	GLL #21	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
106	MF# 5467	GLL #22	Emydinae	Pseudemys	texana		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
107	MF# 5468	GLL #23	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
108	MF# 5469	GLL #24	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
109	MF# 5495	GLL # 139	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/30/02
110	MF# 5496	GLL # 140	Emydinae	Pseudemys	texana		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/30/02
111	MF# 5797	GLL #318	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
112	MF# 5798	GLL #319	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
113	MF# 5800	GLL #321	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
114	MF# 5801	GLL #322	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
115	MF# 5802	GLL #323	Emydinae	Pseudemys	texana		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
116	MF# 5803	GLL #324	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
117	MF# 5804	GLL #325	Emydinae	Pseudemys	texana		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
118	MF# 5810	GLL #331	Emydinae	Pseudemys	texana		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/04/02
119	MF# 5856	GLL #377	Emydinae	Pseudemys	texana		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02

	A	M	N	O	P	Q	R	S	T	U	V
1	MF #	Additional Data	PlastronLength/SVL	PlastronWidth/TL	CarapaceLength	CarapaceWidth	Wgt	W/C/WCCR	% parasitemia	parasite mmts (microns)	PCR test
61	MF# 5832	HG82, 3 mo Captive	n/a	n/a	n/a	n/a	3560 g	W	0	n/a	neg
62	MF# 5833	HG92, 3 mo Captive	n/a	n/a	n/a	n/a	3040 g	W	0 48	12 x 4	
63	MF# 5834	HG 88, 3 mo Captive	n/a	n/a	n/a	n/a	4420 g	W	0 12	15 x 4	
64	MF# 5835	HG 59, 3 mo Captive	n/a	n/a	n/a	n/a	n/a	W	1 17	14 x 6	pos
65	MF#7952	HG87	n/a	n/a	n/a	n/a	n/a	W	0 64	14 x 6	pos
66	MF# 5826	HA6, 3 mo Captive	n/a	n/a	n/a	n/a	5780 g	W	0 61	12 x 4	pos
67	MF# 5827	HA21, 3 mo Captive	n/a	n/a	n/a	n/a	4160 g	W	0 09	12 x 6	
68	MF# 5511	wild born, 5 yr Captive	115 mm	59 mm	n/a	n/a	260 g	WCCR	0 62	0 62	
69	MF# 5513	wild born, 5 yr Captive	112 mm	57 mm	n/a	n/a	260 g	WCCR	0 03	14 x 5	pos
70	MF# 5514	wild born, 5 yr Captive	139 mm	73 mm	n/a	n/a	460 g	WCCR	0	n/a	neg
71	MF# 5484	captive born, 2 yr	n/a	n/a	n/a	n/a	533 g	C	0	n/a	neg
72	MF# 5485	captive born, 2 yr	n/a	n/a	n/a	n/a	315 g	C	0	n/a	
73	MF#5486	captive born, 2 yr	n/a	n/a	n/a	n/a	353 g	C	0	n/a	neg
74	MF# 5487	captive born, 2 yr	n/a	n/a	n/a	n/a	273 g	C	0	n/a	
75	MF# 5456		n/a	n/a	n/a	n/a	1 74 kg	W	2 06	12-14 x 6-7	
76	MF# 5457		n/a	n/a	n/a	n/a		W	2 51	10-19 x 6-10	
77	MF#5454		n/a	n/a	n/a	n/a	3 68 kg	W	0 64	8-14 x 3-8	
78	MF# 5455		n/a	n/a	n/a	n/a		W	0	n/a	neg
79	MF# 5815	OB74, 3 month captive	n/a	n/a	n/a	n/a	1740 g	W	3 42	20 x 8	pos
80	MF# 5816	OB55, 3 month captive	n/a	n/a	n/a	n/a	2160 g	W	0 02	16 x 4	
81	MF# 5824	OB76, 3 mo Captive	n/a	n/a	n/a	n/a	3340 g	W	0 48	10 x 5	
82	MF#7947	OB81	n/a	n/a	n/a	n/a		W	0 62	10 x 3	pos
83	MF# 7948	OB110, 3 mo Captive	n/a	n/a	n/a	n/a	6960 g	W	0 71	12 x 6	pos
84	MF#7949	OB15	n/a	n/a	n/a	n/a		W	0 62	10 x 3	pos
85	MF# 5507	wild born, 5 yr Captive	155 mm	105 mm	n/a	n/a	800 g	WCCR	0	n/a	neg
86	MF# 5516	wild born, 5 yr Captive	174 mm	121 mm	n/a	n/a	1220	WCCR	no slides	n/a	
87	MF# 5519	wild born, 5 yr Captive	162 mm	127 mm	n/a	n/a	700 g	WCCR	0	n/a	neg
88	MF# 5517	wild born, 4 yr Captive	155 mm	122 mm	n/a	n/a	600 g	WCCR	0	n/a	neg
89	MF# 5520	wild born, 4 yr Captive	179 mm	128 mm	n/a	n/a	1100 g	WCCR	0	n/a	neg
90	MF# 5524	wild born, 4 yr Captive	141 mm	104 mm	n/a	n/a	540 g	WCCR	0	n/a	
91	MF# 5853	pit#036-843-043	n/a	n/a	n/a	n/a	1140 g	WCCR	0	n/a	neg
92	MF# 5854	2 drills on left side	n/a	n/a	n/a	n/a	1260 g	WCCR	0	n/a	
93	MF# 5855	notched left	n/a	n/a	n/a	n/a	780 g	WCCR	0	n/a	
94	MF# 5530	wild born, 3 yr Captive	120 mm	83 mm	n/a	n/a	220 g	WCCR	0	n/a	neg
95	MF# 5531	wild born, 3 yr Captive	134 mm	85 mm	n/a	n/a	280 g	WCCR	0	n/a	
96	MF# 5796	RM1 LG2, pit#031-588-600	207 mm	n/a	218 mm	175 mm	1300 g	W	0 06	6 x 3	pos
97	MF# 5799	LM 12 LG 2	149 mm	n/a	160 mm	126 mm	700 g	W	0 039	12 x 4	
98	MF# 5459	LM1 RM1 RA, pit#031-582-323	253 mm	n/a	289 mm	217 mm	2800 g	W	0 11	13 5 x 6	pos
99	MF# 5460	LM1 RM5 LG2, pit#031-580-027	181 mm	n/a	201 mm	148 mm	800 g	W	0 16	13 x 6	neg
100	MF# 5461	LM1,2 RM6 LG2	155 mm	n/a	178 mm	128 mm	675 g	W	0 21	12 5 x 6	pos
101	MF# 5462	LM5 RM10 LA	154 mm	n/a	176 mm	134 mm	650 g	W	0 02	14 x 7	pos
102	MF# 5463	LM1,2 RM7 LG2	151 mm	n/a	175 mm	135 mm	625 g	W	0 01	12 x 4	neg
103	MF# 5464	LM1,2 RM8 LG2	150 mm	n/a	177 mm	131 mm	575 g	W	0 25	12-14 x 5-6	neg
104	MF# 5465	LM1,2 RM12 LG2	182 mm	n/a	211 mm	154 mm	1100 g	W	0 01	12 x 5	neg
105	MF# 5466	LM1,2 RM9 LG2	181 mm	n/a	210 mm	155 mm	1050 g	W	0 23	8-10 x 4	pos
106	MF# 5467	LM1,2 RM10 LG2 pit# 031-582-882	183 mm	n/a	213 mm	158 mm	1200 g	W	0 15	10 x 6	pos
107	MF# 5468	LM1,2 RM11 LG2	146 mm	n/a	177 mm	127 mm	575 g	W	0 09	13 x 4-5 5	pos
108	MF# 5469	LM1,2 RM4	149 mm	n/a	165 mm	130 mm	600 g	W	0 11	13 x 6	pos
109	MF# 5495	LM2,9 RM 2, RH pit # 025-363-367	184 mm	n/a	217 mm	157 mm	1000 g	W	0 03	8-12 x 3-4	neg
110	MF# 5496	LM 1,3 RM 4 LG 2	250 mm	n/a	274 mm	204 mm	n/a	W	0 08	14 x 4	pos
111	MF# 5797	LM1,3 RM8 (accidental 11), LG2	187 mm	n/a	225 mm	167 mm	1350 g	W	0 07	12-13 x 4-6	
112	MF# 5798	LM1,3 RM10,12 LG2	136 mm	n/a	154 mm	119 mm	500 g	W	0 01	8-11 x 3-4	
113	MF# 5800	LM1,3 RM6 LG2	210 mm	n/a	241mm	171 mm	1600 g	W	0 05	12 5 x 4	
114	MF# 5801	LM1,3 RM8 LG2	172 mm	n/a	205 mm	147 mm	1000 g	W	0 04	12 x 4	
115	MF# 5802	LM1,3 RM7 (12 missing) LG2, pit#031-585-033	209 mm	n/a	233 mm	177 mm	1750 g	W	0 17	6-12 x 4	
116	MF# 5803	LM1,3 RM9 LG2	145 mm	n/a	159 mm	123 mm	600 g	W	0 11	6-10 x 3-6	
117	MF# 5804	LM1,3 RM6 LG2	210 mm	n/a	241 mm	171 mm	1600 g	W	0 03	8 x 4	
118	MF# 5810	Lm1,3 RM12 LG2, pit#031-581-809	234 mm	n/a	261 mm	190 mm	2100 g	W	0 63	12 x 4	pos
119	MF# 5856	LM1,4 RM2 LG2, pit#031-591-870	211 mm	n/a	230 mm	178 mm	1600 g	W	0 13	13 x 2-5	

	A	B	C	D	E	F	G	H	I	J	K	L
1	MF #	Collector #	Family	*Genus*	*Species*	*Subspecies*	Sex	Specific Locality	County	State	Country	Date Collected
120	MF# 5857	GLL #378	Emydinae	*Pseudemys*	*texana*		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
121	MF# 5858	GLL #379	Emydinae	*Pseudemys*	*texana*		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
122	MF# 5859	GLL #380	Emydinae	*Pseudemys*	*texana*		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
123	MF# 5860	GLL #381	Emydinae	*Pseudemys*	*texana*		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
124	MF# 5866	GLL #387	Emydinae	*Pseudemys*	*texana*		F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
125	MF# 5881	GLL #402	Emydinae	*Pseudemys*	*gorzugi*		F	Oasis Ranch, West Texas		TX	USA	04/13/02
126	MF# 5882	GLL #403	Emydinae	*Pseudemys*	*gorzugi*		M	Oasis Ranch, West Texas		TX	USA	04/13/02
127	MF# 5883	GLL #404	Emydinae	*Pseudemys*	*gorzugi*		M	Oasis Ranch, West Texas		TX	USA	04/13/02
128	MF# 5884	GLL #405	Emydinae	*Pseudemys*	*gorzugi*		M	Oasis Ranch, West Texas		TX	USA	04/14/02
129	MF# 5498	GLL # 142	Emydinae	*Pseudemys*	*texana*		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/30/02
130	MF# 5499	GLL # 143	Emydinae	*Pseudemys*	*texana*		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/30/02
131	MF# 5500	GLL # 144	Emydinae	*Pseudemys*	*texana*		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/30/02
132	MF# 5501	GLL # 145	Emydinae	*Pseudemys*	*texana*		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/30/02
133	MF# 5868	GLL #389	Emydinae	*Pseudemys*	*gorzugi*		M	Oasis Ranch, West Texas		TX	USA	04/12/02
134	MF# 5869	GLL #390	Emydinae	*Pseudemys*	*gorzugi*		M	Oasis Ranch, West Texas		TX	USA	04/12/02
135	MF# 5870	GLL #391	Emydinae	*Pseudemys*	*gorzugi*		M	Oasis Ranch, West Texas		TX	USA	04/12/02
136	MF# 5871	GLL #392	Emydinae	*Pseudemys*	*gorzugi*		F	Oasis Ranch, West Texas		TX	USA	04/12/02
137	MF# 5875	GLL #396	Emydinae	*Pseudemys*	*gorzugi*		F	Oasis Ranch, West Texas		TX	USA	04/13/02
138	MF# 5876	GLL #397	Emydinae	*Pseudemys*	*gorzugi*		F	Oasis Ranch, West Texas		TX	USA	04/13/02
139	MF# 5877	GLL #398	Emydinae	*Pseudemys*	*gorzugi*		M	Oasis Ranch, West Texas		TX	USA	04/13/02
140	MF# 5878	GLL #399	Emydinae	*Pseudemys*	*gorzugi*		M	Oasis Ranch, West Texas		TX	USA	04/13/02
141	MF# 5879	GLL #400	Emydinae	*Pseudemys*	*gorzugi*		M	Oasis Ranch, West Texas		TX	USA	04/13/02
142	MF# 5504	GLL # 148	Batagurıdae	*Rhinoclemmys*	*punctularia*		M	Guthrie turtle farms		AL	USA	03/14/02
143	MF# 5522	GLL # 166	Batagurıdae	*Sacalia*	*bealeyi*		M	Guthrie turtle farms		AL	USA	03/14/02
144	MF# 5523	GLL # 167	Batagurıdae	*Sacalia*	*bealeyi*		F	Guthrie turtle farms		AL	USA	03/14/02
145	MF# 5458	GLL #5	Batagurıdae	*Siebenrockiella*	*crassicollis*			Waterlife	Hays	TX	USA	01/18/02
146	MF# 5508	GLL # 152	Batagurıdae	*Siebenrockiella*	*crassicollis*		F	Guthrie turtle farms		AL	USA	03/14/02
147	MF# 5509	GLL # 153	Batagurıdae	*Siebenrockiella*	*crassicollis*		F	Guthrie turtle farms		AL	USA	03/14/02
148	MF# 5505	GLL # 149	Batagurıdae	*Siebenrockiella*	*crassicollis*		M	Guthrie turtle farms		AL	USA	03/14/02
149	MF# 5506	GLL # 150	Batagurıdae	*Siebenrockiella*	*crassicollis*		M	Guthrie turtle farms		AL	USA	03/14/02
150	MF# 5510	GLL # 154	Batagurıdae	*Siebenrockiella*	*crassicollis*		F	Guthrie turtle farms		AL	USA	03/14/02
151	MF#7951	GLL#439	Batagurıdae	*Siebenrockiella*	*crassicollis*		M	Waterlife	Hays	TX	USA	
152	MF#7950	GLL#438	Batagurıdae	*Siebenrockiella*	*crassicollis*		F	Waterlife	Hays	TX	USA	
153	MF# 5515	GLL # 159	Kinosternıdae	*Staurotypus*	*triporcatus*		F	Guthrie turtle farms		AL	USA	03/14/02
154	MF# 5521	GLL # 165	Kinosternıdae	*Staurotypus*	*triporcatus*		F	Guthrie turtle farms		AL	USA	03/14/02
155	MF# 5852	GLL #373	Kinosternıdae	*Staurotypus*	*triporcatus*		F	Waterlife	Hays	TX	USA	04/06/02
156	MF# 5851	GLL #372	Kinosternıdae	*Staurotypus*	*triporcatus*		F	Waterlife	Hays	TX	USA	04/06/02
157	MF# 5527	GLL # 171	Kinosternıdae	*Staurotypus*	*triporcatus*		F	Guthrie turtle farms		AL	USA	03/14/02
158	MF# 5528	GLL # 172	Kinosternıdae	*Staurotypus*	*triporcatus*		M	Guthrie turtle farms		AL	USA	03/14/02
159	MF# 5529	GLL # 173	Kinosternıdae	*Staurotypus*	*triporcatus*		M	Guthrie turtle farms		AL	USA	03/14/02
160	GLL#26	GLL#26	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	09/24/01
161	GLL#27	GLL#27	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/07/01
162	GLL#28	GLL#28	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/07/01
163	GLL#29	GLL#29	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/07/01
164	GLL#30	GLL#30	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/07/01
165	GLL#31	GLL#31	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/07/01
166	GLL#32	GLL#32	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/07/01
167	GLL#33	GLL#33	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/07/01
168	GLL#34	GLL#34	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/07/01
169	GLL#35	GLL#35	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/07/01
170	GLL#36	GLL#36	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/07/01
171	GLL#37	GLL#37	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/10/01
172	GLL#38	GLL#38	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/10/01
173	GLL#39	GLL#39	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/10/01
174	GLL#40	GLL#40	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/10/01
175	GLL#41	GLL#41	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/10/01
176	GLL#42	GLL#42	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/10/01
177	GLL#43	GLL#43	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/10/01
178	GLL#44	GLL#44	Kinosternıdae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/10/01

	A	M	N	O	P	Q	R	S	T	U	V
1	MF #	Additional Data	PlastronLength/SVL	PlastronWidth/TL	CarapaceLength	CarapaceWidth	Wgt	W/C/WCCR	% parasitemia	parasite mmts (microns)	PCR test
120	MF# 5857	LM1,4 RM3, LG2, pit#031-601-355	233 mm	n/a	233 mm	176 mm	1850 g	W	0	n/a	neg
121	MF# 5858	LM1,4 RM4, LG2, pit#031-589-028	240 mm	n/a	271 mm	212 mm	2600 g	W	0	n/a	neg
122	MF# 5859	LM1,4 RM5, LG2, pit#031-586-352	235 mm	n/a	256 mm	201 mm	2700 g	W	0 05	12 x 4	
123	MF# 5860	LM6 RM2,12 LA	160 mm	n/a	176 mm	136 mm	700 g	W	0 38	12 x 5	
124	MF# 5866	LM1,4 RM1 LG2, pit#031-591-870	226 mm	n/a	246 mm	184 mm	2100 g	W	0 21	12 x 4	
125	MF# 5881	PG10	59 mm	49 mm	67 mm	60 mm	60 g	W	0	n/a	
126	MF# 5882	PG11	154 mm	103 mm	174 mm	129 mm	620 g	W	0	n/a	neg
127	MF# 5883	(rt front flipper removed, old wound, radius and ulna protruding)	141 mm	94 mm	162 mm	124 mm	540 g	W	0	n/a	neg
128	MF# 5884	PG12	167 mm	109 mm	193 mm	136 mm	900 g	W	0	n/a	
129	MF# 5498	LM 1,2 RM 1 LG 2	176 mm	n/a	206 mm	156 mm	950 g	W	0 1	11 x 5	
130	MF# 5499	pit # 031-588-812	206 mm	n/a	243 mm	177 mm	1650 g	W	0 39	8 x 4	
131	MF# 5500	LG 1,2 RM 2 LG 2	169 mm	n/a	198 mm	149 mm	850 g	W	0 1	13 x 6	
132	MF# 5501	LM 1,3 RM3 LG2	141 mm	n/a	168 mm	125 mm	525 g	W	0 28	13 x 6	
133	MF# 5868	PG2 (drilled #2 according to IUCN field form)	210 mm	130 mm	220 mm	162 mm	1440 g	W	0	n/a	
134	MF# 5869	PG4 (drilled #4 according to IUCN field form)	202 mm	140 mm	246 mm	175 mm	1840 g	W	0	n/a	
135	MF# 5870	PG3 (drilled #3 according to IUCN field form)	187 mm	118 mm	260 mm	151 mm	1180 g	W	0	n/a	neg
136	MF# 5871	PG1 (drilled #1 according to IUCN field form)	236 mm	157 mm	261 mm	196 mm	2520 g	W	0	n/a	
137	MF# 5875	PG5	268 mm	181 mm	296 mm	260 mm	3820 g	W	0	n/a	neg
138	MF# 5876	PG6	235 mm	150 mm	264 mm	181 mm	2600 g	W	0	n/a	
139	MF# 5877	PG7	190 mm	132 mm	220 mm	164 mm	1360 g	W	0	n/a	neg
140	MF# 5878	PG8	155 mm	101 mm	126 mm	132 mm	660 g	W	0	n/a	neg
141	MF# 5879	PG9	168 mm	112 mm	183 mm	142 mm	880 g	W	0	n/a	
142	MF# 5504	wild born, 4 yr Captive	140 mm	110 mm	n/a	n/a	760 g	WCCR	0	n/a	neg
143	MF# 5522	wild born, 4 yr Captive	116 mm	87 mm	n/a	n/a	200 g	WCCR	no slides	n/a	neg
144	MF# 5523	wild born, 4 yr Captive	128 mm	82 mm	n/a	n/a	260 g	WCCR	no slides	n/a	neg
145	MF# 5458	(from Malaysia)	n/a	n/a	n/a	n/a	1 16 kg	W	0 92	13 x 5-7	
146	MF# 5508	wild born, 3 month Captive	137 mm	111 mm	n/a	n/a	740 g	W	0 01	n/a	
147	MF# 5509	wild born, 3 month Captive	153 mm	115 mm	n/a	n/a	840 g	W	0 05	n/a	
148	MF# 5505	wild born, 3 yr Captive	132 mm	105 mm	n/a	n/a	720 g	WCCR	0	n/a	neg
149	MF# 5506	wild born, 3 yr Captive	136 mm	110 mm	n/a	n/a	720 g	WCCR	0	n/a	neg
150	MF# 5510	wild born, 3 month Captive	134 mm	113 mm	n/a	n/a	700 g	W	1 75	n/a	pos
151	MF#7951	SC124	n/a	n/a	n/a	n/a	n/a	W	1 02	n/a	pos
152	MF#7950	SC82	n/a	n/a	n/a	n/a	n/a	W	0 85	n/a	
153	MF# 5515	wild born, 7 yr Captive	n/a	n/a	n/a	n/a	4140 g	WCCR	0	n/a	neg
154	MF# 5521	wild born, 7 yr Captive	n/a	n/a	n/a	n/a	n/a	WCCR	0	n/a	neg
155	MF# 5852	captive born 1998	n/a	n/a	n/a	n/a	1480 g	C	0	n/a	
156	MF# 5851	captive born 1998, exposed to leeches by wild caught P texana	n/a	n/a	n/a	n/a	800 g	WCCR	0	n/a	
157	MF# 5527	captive born, 1 yr	49 mm	60 mm	n/a	n/a	80 g	C	no slides	n/a	
158	MF# 5528	captive born, 1 yr	82 mm	80 m	n/a	n/a	320 g	C	0	n/a	neg
159	MF# 5529	captive born, 1 yr	74 mm	71 mm	n/a	n/a	340 g	C	0	n/a	neg
160	GLL#26	LM7 RM9 =	71 6	n/a	71 6	48 6	52 6	W	0 2	10 x 4	
161	GLL#27	LM2 RM4 =	88 8	n/a	88 8	60 1	103 9	W	0	n/a	
162	GLL#28	LM1,2 RM11 -	68 2	n/a	68 2	49 8	48 5	W	0 24	10 x 4	
163	GLL#29	LM2 RM5 =	93 8	n/a	93 8	58 7	119 7	W	0 02	10 x 5	
164	GLL#30	LM4,7 RM3 -	92 2	n/a	92 2	61 6	146	W	0	n/a	
165	GLL#31	LM2 RM2 =	93 7	n/a	93 7	61 8	128 2	W	0	n/a	
166	GLL#32	LM1,4,11 RM2 -	n/a	n/a	n/a	n/a	n/a	W	0	n/a	
167	GLL#33	LM2 RM3	81 7	n/a	81 7	54 4	82 3	W	0 15	13 x 6	
168	GLL#34	LM1 RM11	82	n/a	82	58	85	W	0 04	9 5 x 3	
169	GLL#35	LM2,1 =	n/a	n/a	n/a	n/a	n/a	W	0 1	9 x 4	
170	GLL#36	LM5, 10-11 ??	85 9	n/a	85 9	54 8	96 4	W	0 2	10 x 5 5	
171	GLL#37	LM2 RM8 =	n/a	n/a	n/a	n/a	n/a	W	0 2	12 x 6	
172	GLL#38	LM5,9 RM4 -	82 3	n/a	82 3	58 8	96 8	W	0 03	10 x 4	
173	GLL#39		n/a	n/a	n/a	n/a	n/a	W	0 13	8 x 4	
174	GLL#40	LM2 RM6 =	79 5	n/a	79 5	54 5	75 2	W	0	n/a	
175	GLL#41	LM2 RM10 =	75	n/a	75	57	75	W	0 1	8 5 x 4 5	
176	GLL#42	LM2 RM11 =	87	n/a	87	53 9	88	W	0 2	11 x 5	
177	GLL#43	LM3 RM10 -	n/a	n/a	n/a	n/a	n/a	W	0 05	8 x 5	
178	GLL#44	LM2 RM9 =	80 9	n/a	80 9	57 1	82 8	W	0 02	9 x 5	

	A	B	C	D	E	F	G	H	I	J	K	L
1	MF #	Collector #	Family	*Genus*	*Species*	*Subspecies*	Sex	Specific Locality	County	State	Country	Date Collected
179	GLL#45	GLL#45	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/10/01
180	GLL#46	GLL#46	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/10/01
181	GLL#47	GLL#47	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
182	GLL#48	GLL#48	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
183	GLL#49	GLL#49	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
184	GLL#50	GLL#50	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
185	GLL#51	GLL#51	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
186	GLL#52	GLL#52	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
187	GLL#53	GLL#53	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
188	GLL#54	GLL#54	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
189	GLL#55	GLL#55	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
190	GLL#56	GLL#56	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
191	GLL#57	GLL#57	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
192	GLL#58	GLL#58	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/25/01
193	GLL#59	GLL#59	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/28/01
194	GLL#60	GLL#60	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/28/01
195	GLL#61	GLL#61	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/28/01
196	GLL#62	GLL#62	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/28/01
197	GLL#63	GLL#63	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/28/01
198	GLL#64	GLL#64	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/28/01
199	GLL#65	GLL#65	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/28/01
200	GLL#66	GLL#66	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	06/28/01
201	GLL#67	GLL#67	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	07/14/01
202	GLL#68	GLL#68	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	07/14/01
203	GLL#69	GLL#69	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	07/14/01
204	GLL#70	GLL#70	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	07/14/01
205	GLL#71	GLL#71	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	07/14/01
206	GLL#72	GLL#72	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	07/14/01
207	GLL#73	GLL#73	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	07/14/01
208	GLL#74	GLL#74	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	07/14/01
209	GLL#75	GLL#75	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
210	GLL#76	GLL#76	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
211	GLL#77	GLL#77	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
212	GLL#78	GLL#78	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
213	GLL#79	GLL#79	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
214	GLL#80	GLL#80	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
215	GLL#81	GLL#81	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
216	GLL#82	GLL#82	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
217	GLL#83	GLL#83	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
218	GLL#84	GLL#84	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
219	GLL#85	GLL#85	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
220	GLL#86	GLL#86	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
221	GLL#87	GLL#87	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
222	GLL#88	GLL#88	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
223	GLL#89	GLL#89	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
224	GLL#90	GLL#90	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
225	GLL#91	GLL#91	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
226	GLL#92	GLL#92	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
227	GLL#93	GLL#93	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
228	GLL#94	GLL#94	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
229	GLL#95	GLL#95	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
230	GLL#96	GLL#96	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
231	GLL#97	GLL#97	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
232	GLL#98	GLL#98	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
233	GLL#99	GLL#99	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
234	GLL#100	GLL#100	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
235	GLL#101	GLL#101	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
236	GLL#102	GLL#102	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
237	GLL#103	GLL#103	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	

	A	M	N	O	P	Q	R	S	T	U	V
1	MF #	Additional Data	PlastronLength/SVL	PlastronWidth/TL	CarapaceLength	CarapaceWidth	Wgt	W/C/WCCR	% parasitemia	parasite mmts (microns)	PCR test
179	GLL#45	LM2 RM7 -	83 7	n/a	83 7	59 4	90 5	W	0 6	10 x 5	
180	GLL#46	LM4 RM3 =	97 5	n/a	97 5	59 8	120 3	W	0 02	11 x 4	
181	GLL#47	LM2,? RM1 -	97 5	n/a	97 5	63 5	138 6	W	0 06	12 5 x 6	
182	GLL#48	LM7,11 RM10 -	91	n/a	91	62 1	120 5	W	0	n/a	
183	GLL#49	LM3 RM4 =	81 9	n/a	81 9	58 6	83 3	W	0 02	12 x 4	
184	GLL#50	LM3 RM5 =	100 2	n/a	100 2	64 1	143 1	W	0 07	12 x 6	
185	GLL#51	LM3 RM8 =	84 2	n/a	84 2	56 8	102 4	W	0 1	10 x 5	
186	GLL#52	LM1,5 RM10 -	83 9	n/a	83 9	52 7	92 8	W	0 1	12 5 x 5	
187	GLL#53	LM2 RM2 =	93 7	n/a	93 7	61 8	128 2	W	0 05	10 x 6	
188	GLL#54	LM3 RM6 =	96 8	n/a	96 8	65 3	137 3	W	0 02	12 x 4	
189	GLL#55	LM3 RM7 =	90 7	n/a	90 7	59 1	101 6	W	0 16	16 5 x 5	
190	GLL#56	?	n/a	n/a	n/a	n/a	n/a	W	0 14	8 x 4	
191	GLL#57	LM11 RM3 -	93 3	n/a	93 3	64 9	122	W	0 2	9 x 4	
192	GLL#58	LM6,10 RM10 -	85 4	n/a	85 4	57 1	91 9	W	0	n/a	
193	GLL#59	LM2,10 RM7	93	n/a	93	63 4	131 2	W	0 14	9 x 4	
194	GLL#60	LM4,6 RM2 -	90 2	n/a	90 2	56 7	95	W	0 07	9 x 5	
195	GLL#61	LM5,7 RM10 -	73 6	n/a	73 6	52 4	77	W	0 08	9 x 4	
196	GLL#62	LM2 RM9 -	81 5	n/a	81 5	57 5	80 7	W	0 01	8 x 4	
197	GLL#63	LM6,7 RM7 -	80 2	n/a	80 2	55 9	83 8	W	0 05	10 x 4	
198	GLL#64	LM4 RM5 =	78 9	n/a	78 9	54	74 5	W	0	n/a	
199	GLL#65	LM5,7 RM11 -	88 2	n/a	88 2	58 2	122 4	W	0 16	12 x 6	
200	GLL#66	LM4 RM10 =	88 2	n/a	88 2	55 6	91 3	W	0 16	10 x 5	
201	GLL#67	LM2,11 RM3 -	81 8	n/a	81 8	54 3	89	W	0	n/a	
202	GLL#68	LM4 RM8 =	63 6	n/a	63 6	46 3	37 4	W	0 1	8-14 x 5-6	
203	GLL#69	LM1,8 RM5 -	90 9	n/a	90 9	56 5	113 9	W	0 11	8 x 5	
204	GLL#70	LM4 RM9 =	85	n/a	85	58	105	W	0 02	6 x 6	
205	GLL#71	LM4 RM11 =	89 1	n/a	89 1	59 5	106	W	0 01	10 x 5	
206	GLL#72	LM4 RM7 =	81 2	n/a	81 2	52 6	71 5	W	0 06	8-12 x 5-7	
207	GLL#73	LM8,11 RM5 -	n/a	n/a	n/a	n/a	n/a	W	0 04	7 x 4	
208	GLL#74	LM8,10 RM4 -	70 8	n/a	100 6	60 4	140 1	W	0 14	8 x 5	
209	GLL#75	LM5 RM2 =	n/a	n/a	n/a	n/a	112	W	0 03	11 x 5	
210	GLL#76	LM5 RM1 =	51 7	n/a	81 9	50 7	86 3	W	0 1	14 x 5	
211	GLL#77	LM6 RM1 =	58 2	n/a	91 5	58 2	106 7	W	0 05	12 x 6	
212	GLL#78	LM3,6 RM8 -	74 9	n/a	99 7	63 5	155 8	W	0 07	12 x 4	
213	GLL#79	LM5,9 RM10 -	59 5	n/a	86 7	56 4	103 1	W	0 45	10 x 4	
214	GLL#80	LM6 RM8 =	50 7	n/a	73 4	51 2	56 4	W	0 01	10 x 5	
215	GLL#81	LM6 RM9 =	n/a	n/a	n/a	n/a	n/a	W	0 01	10 5 x 5	
216	GLL#82	LM6 RM11 =	58 7	n/a	86 7	56 6	95 9	W	0 06	10 x 6	
217	GLL#83	LM6 RM10 =	55 6	n/a	87 2	55	103 2	W	0 02	12 x 8	
218	GLL#84	LM2,7 RM8 -	57 8	n/a	98 5	56 9	115 5	W	0 03	10 x 4	
219	GLL#85	LM3,6 RM10 -	73 5	n/a	93 5	63 5	140 6	W	0 15	14 x 5	
220	GLL#86	NO DATA	n/a	n/a	n/a	n/a	n/a	W	0 1	12 x 4 5	
221	GLL#87	LM7 RM2 =	58	n/a	90 6	59 2	106 2	W	0 03	7 x 3	
222	GLL#88	LM1,4,11 RM1 -	n/a	n/a	n/a	n/a	n/a	W	0 04	10 x 5	
223	GLL#89	LM7 RM2 =	58	n/a	90 6	59 2	106 2	W	0 01	12 x 5	
224	GLL#90	LM8,11 RM6 -	n/a	n/a	n/a	n/a	n/a	W	0 05	8 x 4	
225	GLL#91	LM7 RM5 =	57 7	n/a	81 8	56 3	82 2	W	0 06	12 x 5	
226	GLL#92	LM1,3,10 RM1 -	n/a	n/a	n/a	n/a	n/a	W	0	n/a	
227	GLL#93	LM7 RM4 =	n/a	n/a	n/a	n/a	110 3	W	0	n/a	
228	GLL#94	LM1,4,10 RM2 -	n/a	n/a	n/a	n/a	n/a	W	0 03	12 x 4	
229	GLL#95	LM7 RM8 =	48 5	n/a	71	49 6	57 2	W	0 17	10 x 3	
230	GLL#96	LM3,4 RM7 -	59 5	n/a	93	50	112 7	W	0	n/a	
231	GLL#97	LM6,7 RM10 -	53 2	n/a	73 1	50 8	71 2	W	0 03	14 x 6	
232	GLL#98	LM7 RM2 =	58	n/a	90 6	59 2	106 2	W	0 02	12 x 4	
233	GLL#99	LM7 RM6 =	55 9	n/a	83 6	56 2	90	W	0 02	10 x 6	
234	GLL#100	LM7 RM10 =	64 9	n/a	101	65 7	147 3	W	0	n/a	
235	GLL#101	LM7 RM7 =	54 9	n/a	80 7	55 1	67 6	W	0 13	13 x 4	
236	GLL#102	LM8 RM1 =	56	n/a	89 2	55 9	96 1	W	0 01	10 x 4	
237	GLL#103	LM2 RM2 =	67 4	n/a	93 7	61 8	128 2	W	0 02	10 x 5	

	A	B	C	D	E	F	G	H	I	J	K	L
1	MF #	Collector #	Family	*Genus*	*Species*	*Subspecies*	Sex	Specific Locality	County	State	Country	Date Collected
238	GLL#104	GLL#104	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
239	GLL#105	GLL#105	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
240	GLL#106	GLL#106	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
241	GLL#107	GLL#107	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
242	GLL#108	GLL#108	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
243	GLL#109	GLL#109	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
244	GLL#110	GLL#110	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
245	GLL#111	GLL#111	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
246	GLL#112	GLL#112	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
247	GLL#113	GLL#113	Kinosternidae	*Sternotherus*	*odoratus*			Aquarena Springs, San Marcos	Hays	TX	USA	
248	MF# 5497	GLL # 141	Kinosternidae	*Sternotherus*	*odoratus*		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/30/02
249	MF#5502	GLL#146	Kinosternidae	*Sternotherus*	*odoratus*		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	
250	MF# 5503	GLL # 147	Kinosternidae	*Sternotherus*	*odoratus*		M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/30/02
251	MF# 5889	GLL #410	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Capitol Aggregate, Marble Falls		TX	USA	05/01/02
252	MF# 5532	GLL # 176	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
253	MF# 5533	GLL # 177	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
254	MF# 5534	GLL # 178	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
255	MF# 5535	GLL # 179	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
256	MF# 5536	GLL # 180	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
257	MF# 5537	GLL # 181	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
258	MF# 5538	GLL # 182	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
259	MF# 5539	GLL # 183	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
260	MF# 5540	GLL # 184	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
261	MF# 5541	GLL # 185	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
262	MF# 5542	GLL # 186	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
263	MF# 5543	GLL # 187	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
264	MF# 5544	GLL # 188	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
265	MF# 5545	GLL # 189	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
266	MF# 5546	GLL # 190	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
267	MF# 5547	GLL # 191	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
268	MF# 5548	GLL # 192	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
269	MF# 5549	GLL # 193	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
270	MF# 5550	GLL # 194	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
271	MF# 5551	GLL # 195	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
272	MF# 5552	GLL # 196	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
273	MF# 5553	GLL # 197	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
274	MF# 5554	GLL # 198	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
275	MF# 5555	GLL # 199	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
276	MF# 5556	GLL # 200	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
277	MF# 5557	GLL # 201	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
278	MF# 5558	GLL # 202	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
279	MF# 5559	GLL # 203	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
280	MF# 5560	GLL # 204	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
281	MF# 5561	GLL # 205	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
282	MF# 5562	GLL # 206	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
283	MF# 5563	GLL # 207	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
284	MF# 5564	GLL # 208	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
285	MF# 5565	GLL # 209	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
286	MF# 5566	GLL # 210	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
287	MF# 5567	GLL # 211	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
288	MF# 5568	GLL # 212	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
289	MF# 5570	GLL # 214	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
290	MF# 5571	GLL # 215	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
291	MF# 5572	GLL # 216	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
292	MF# 5573	GLL # 217	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
293	MF# 5574	GLL # 218	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
294	MF# 5575	GLL # 219	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
295	MF# 5576	GLL # 220	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
296	MF# 5577	GLL # 221	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02

	A	M	N	O	P	Q	R	S	T	U	V
1	MF #	Additional Data	PlastronLength/SVL	PlastronWidth/TL	CarapaceLength	CarapaceWidth	Wgt	W/C/WCCR	% parasitemia	parasite mmts (microns)	PCR test
238	GLL#104	LM8 RM3 =	57	n/a	90	57 7	110 1	W	0	n/a	
239	GLL#105	LM1,3,5 RM5? -	n/a	n/a	n/a	n/a	n/a	W	0 05	13 x 3	
240	GLL#106	LM9 RM11 =	66 4	n/a	88 7	60 9	101 3	W	0	n/a	
241	GLL#107	LM9 RM2 =	70 9	n/a	96 9	60	122 4	W	0	n/a	
242	GLL#108	LM9 RM4 =	57 1	n/a	84 4	54 2	84 3	W	0 01	12 x 4	
243	GLL#109	LM9 RM5 =	51 8	n/a	73 4	51 7	54 9	W	0 01	10 x 5	
244	GLL#110	LM3,4 RM10 -	49 6	n/a	71 1	48 2	54	W	0 05	10 x 5	
245	GLL#111	LM9 RM3 =	58	n/a	90 1	56 4	100 7	W	0 02	10 x 5	
246	GLL#112	LM9 RM6 =	49 9	n/a	76 6	51 4	70 5	W	0 02	10 x 4	
247	GLL#113	LM9 RM7 =	52 8	n/a	78 1	51 9	69 9	W	0	n/a	
248	MF# 5497	LM 2,6 RM 6	n/a	n/a	n/a	n/a	n/a	W	0 14	9 x 4	neg
249	MF#5502		n/a	n/a	n/a	n/a	n/a	W	0 16	12 x 4	neg
250	MF# 5503	LM 3,6 =	n/a	n/a	n/a	n/a	n/a	W	0 03	8-10 x 4-5	
251	MF# 5889		240 mm	157 mm	254 mm	200 mm	5 5 lbs	W	0 09	14 x 6	
252	MF# 5532	no markings, dense population	205 mm	134 mm	n/a	n/a	1700 g	C	0	n/a	neg
253	MF# 5533	no markings, dense population	212 mm	138 mm	n/a	n/a	1700 g	C	0	n/a	neg
254	MF# 5534	no markings, dense population	180 mm	125 mm	n/a	n/a	1200 g	C	0	n/a	neg
255	MF# 5535	no markings, dense population	207 mm	131 mm	n/a	n/a	1680 g	C	0	n/a	neg
256	MF# 5536	no markings, dense population	196 mm	125 mm	n/a	n/a	1520 g	C	0	n/a	neg
257	MF# 5537	no markings, dense population	214 mm	136 mm	n/a	n/a	2180 g	C	0	n/a	
258	MF# 5538	no markings, dense population	189 mm	126 mm	n/a	n/a	1440 g	C	0	n/a	
259	MF# 5539	no markings, dense population	173 mm	110 mm	n/a	n/a	1120 g	C	0	n/a	
260	MF# 5540	no markings, dense population	208 mm	127 mm	n/a	n/a	1820 g	C	0	n/a	
261	MF# 5541	no markings, dense population	209 mm	138 mm	n/a	n/a	1940 g	C	0	n/a	
262	MF# 5542	no markings, dense population	175 mm	122 mm	n/a	n/a	1380 g	C	0	n/a	
263	MF# 5543	no markings, dense population	177 mm	109 mm	n/a	n/a	1060 g	C	0	n/a	
264	MF# 5544	no markings, dense population	163 mm	110 mm	n/a	n/a	1020 g	C	0	n/a	
265	MF# 5545	no markings, dense population	212 mm	126 mm	n/a	n/a	1800 g	C	0	n/a	
266	MF# 5546	no markings, dense population	116 mm	78 mm	n/a	n/a	280 g	C	0	n/a	
267	MF# 5547	no markings, dense population	106 mm	79 mm	n/a	n/a	200 g	C	0	n/a	
268	MF# 5548	no markings, dense population	119 mm	81 mm	n/a	n/a	280 g	C	0	n/a	
269	MF# 5549	no markings, dense population	100 mm	71 mm	n/a	n/a	200 g	C	0	n/a	
270	MF# 5550	no markings, dense population	112 mm	83 mm	n/a	n/a	300 g	C	0	n/a	
271	MF# 5551	no markings, dense population	112 mm	81 mm	n/a	n/a	260 g	C	0	n/a	
272	MF# 5552	no markings, dense population	106 mm	78 mm	n/a	n/a	240 g	C	0	n/a	
273	MF# 5553	no markings, dense population	123 mm	91 mm	n/a	n/a	320 g	C	0	n/a	
274	MF# 5554	no markings, dense population	90 mm	77 mm	n/a	n/a	180 g	C	0	n/a	
275	MF# 5555	no markings, dense population	102 mm	78 mm	n/a	n/a	220 g	C	0	n/a	
276	MF# 5556	no markings, dense population	101 mm	82 mm	n/a	n/a	220 g	C	0	n/a	
277	MF# 5557	no markings, dense population	89 mm	69 mm	n/a	n/a	140 g	C	0	n/a	
278	MF# 5558	no markings, dense population	99 mm	74 mm	n/a	n/a	200 g	C	0	n/a	
279	MF# 5559	no markings, dense population	97 mm	69 mm	n/a	n/a	180 g	C	0	n/a	
280	MF# 5560	no markings, dense population	93 mm	68 mm	n/a	n/a	180 g	C	0	n/a	
281	MF# 5561	no markings, dense population	82 mm	63 mm	n/a	n/a	140 g	C	0	n/a	
282	MF# 5562	no markings, dense population	96 mm	72 mm	n/a	n/a	180 g	C	0	n/a	
283	MF# 5563	no markings, dense population	81 mm	66 mm	n/a	n/a	140 g	C	0	n/a	
284	MF# 5564	no markings, dense population	83 mm	61 mm	n/a	n/a	100 g	C	0	n/a	
285	MF# 5565	no markings, dense population	77 mm	62 mm	n/a	n/a	100 g	C	0	n/a	
286	MF# 5566	no markings, dense population	79 mm	59 mm	n/a	n/a	100 g	C	0	n/a	
287	MF# 5567	no markings, dense population	84 mm	65 mm	n/a	n/a	140 g	C	0	n/a	
288	MF# 5568	no markings, dense population	64 mm	49 mm	n/a	n/a	80 g	C	0	n/a	
289	MF# 5570	no markings, dense population	265 mm	163 mm	n/a	n/a	3220 g	C	0	n/a	
290	MF# 5571	no markings, dense population	220 mm	146 mm	n/a	n/a	2020 g	C	0	n/a	
291	MF# 5572	no markings, dense population	241 mm	141 mm	n/a	n/a	2560 g	C	0	n/a	
292	MF# 5573	no markings, dense population	216 mm	133 mm	n/a	n/a	1800 g	C	0	n/a	
293	MF# 5574	no markings, dense population	201 mm	140 mm	n/a	n/a	1920 g	C	0	n/a	
294	MF# 5575	no markings, dense population	211 mm	136 mm	n/a	n/a	2000 g	C	0	n/a	
295	MF# 5576	no markings, dense population	238 mm	142 mm	n/a	n/a	1960 g	C	0	n/a	
296	MF# 5577	no markings, dense population	210 mm	133 mm	n/a	n/a	1880 g	C	0	n/a	

	A	B	C	D	E	F	G	H	I	J	K	L
1	MF #	Collector #	Family	*Genus*	*Species*	*Subspecies*	Sex	Specific Locality	County	State	Country	Date Collected
297	MF# 5578	GLL # 222	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
298	MF# 5579	GLL # 223	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
299	MF# 5580	GLL # 224	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
300	MF# 5581	GLL # 225	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
301	MF# 5582	GLL # 226	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
302	MF# 5583	GLL # 227	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
303	MF# 5584	GLL # 228	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
304	MF# 5585	GLL # 229	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
305	MF# 5586	GLL # 230	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
306	MF# 5587	GLL # 231	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
307	MF# 5588	GLL # 232	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
308	MF# 5589	GLL # 233	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
309	MF# 5590	GLL # 234	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
310	MF# 5591	GLL # 235	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
311	MF# 5592	GLL # 236	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
312	MF# 5593	GLL # 237	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
313	MF# 5594	GLL # 238	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
314	MF# 5595	GLL # 239	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
315	MF# 5596	GLL # 240	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
316	MF# 5597	GLL # 241	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
317	MF# 5598	GLL # 242	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
318	MF# 5599	GLL # 243	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
319	MF# 5600	GLL # 244	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
320	MF# 5601	GLL # 245	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
321	MF# 5602	GLL # 246	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
322	MF# 5603	GLL # 247	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
323	MF# 5604	GLL # 248	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
324	MF# 5605	GLL # 249	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
325	MF# 5606	GLL # 250	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
326	MF# 5607	GLL # 251	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
327	MF# 5608	GLL # 252	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
328	MF# 5609	GLL # 253	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
329	MF# 5610	GLL # 254	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
330	MF# 5611	GLL # 255	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
331	MF# 5612	GLL # 256	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
332	MF# 5613	GLL # 257	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
333	MF# 5614	GLL # 258	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
334	MF# 5615	GLL # 259	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
335	MF# 5616	GLL # 260	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
336	MF# 5617	GLL # 261	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
337	MF# 5618	GLL # 262	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
338	MF# 5619	GLL # 263	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
339	MF# 5620	GLL # 264	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
340	MF# 5621	GLL # 265	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
341	MF# 5622	GLL # 266	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
342	MF# 5623	GLL # 267	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
343	MF# 5624	GLL # 268	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
344	MF# 5625	GLL # 269	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
345	MF# 5626	GLL # 270	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
346	MF# 5627	GLL # 271	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
347	MF# 5628	GLL # 272	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
348	MF# 5629	GLL # 273	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
349	MF# 5630	GLL # 274	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
350	MF# 5631	GLL # 275	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
351	MF# 5632	GLL # 276	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
352	MF# 5633	GLL # 277	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
353	MF# 5634	GLL # 278	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
354	MF# 5635	GLL # 279	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
355	MF# 5636	GLL # 280	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02

	A	M	N	O	P	Q	R	S	T	U	V
1	MF #	Additional Data	PlastronLength/SVL	PlastronWidth/TL	CarapaceLength	CarapaceWidth	Wgt	W/C/WCCR	% parasitemia	parasite mmts (microns)	PCR test
297	MF# 5578	no markings, dense population	209 mm	142 mm	n/a	n/a	1900 g	C	0	n/a	
298	MF# 5579	no markings, dense population	223 mm	131 mm	n/a	n/a	2020 g	C	0	n/a	
299	MF# 5580	no markings, dense population	192 mm	119 mm	n/a	n/a	1340 g	C	0	n/a	
300	MF# 5581	no markings, dense population	201 mm	123 mm	n/a	n/a	1580 g	C	0	n/a	
301	MF# 5582	no markings, dense population	230 mm	145 mm	n/a	n/a	2200 g	C	0	n/a	
302	MF# 5583	no markings, dense population	205 mm	120 mm	n/a	n/a	1740 g	C	0	n/a	
303	MF# 5584	no markings, dense population	207 mm	128 mm	n/a	n/a	1600 g	C	0	n/a	
304	MF# 5585	no markings, dense population	221 mm	132 mm	n/a	n/a	2040 g	C	0	n/a	
305	MF# 5586	no markings, dense population	192 mm	124 mm	n/a	n/a	1300 g	C	0	n/a	
306	MF# 5587	no markings, dense population	205 mm	131 mm	n/a	n/a	1580 g	C	0	n/a	
307	MF# 5588	no markings, dense population	211 mm	130 mm	n/a	n/a	1760 g	C	0	n/a	
308	MF# 5589	no markings, dense population	239 mm	133 mm	n/a	n/a	2280 g	C	0	n/a	
309	MF# 5590	no markings, dense population	221 mm	143 mm	n/a	n/a	1920 g	C	0	n/a	
310	MF# 5591	no markings, dense population	221 mm	131 mm	n/a	n/a	1660 g	C	0	n/a	
311	MF# 5592	no markings, dense population	215 mm	132 mm	n/a	n/a	1880 g	C	0	n/a	
312	MF# 5593	no markings, dense population	206 mm	143 mm	n/a	n/a	1560 g	C	0	n/a	
313	MF# 5594	no markings, dense population	213 mm	135 mm	n/a	n/a	1780 g	C	0	n/a	
314	MF# 5595	no markings, dense population	215 mm	132 mm	n/a	n/a	1560 g	C	0	n/a	
315	MF# 5596	no markings, dense population	225 mm	142 mm	n/a	n/a	2220 g	C	0	n/a	
316	MF# 5597	no markings, dense population	204 mm	129 mm	n/a	n/a	1700 g	C	0	n/a	
317	MF# 5598	no markings, dense population	229 mm	141 mm	n/a	n/a	2160 g	C	0	n/a	
318	MF# 5599	no markings, dense population	199 mm	119 mm	n/a	n/a	1500 g	C	0	n/a	
319	MF# 5600	no markings, dense population	208 mm	137 mm	n/a	n/a	2080 g	C	0	n/a	
320	MF# 5601	no markings, dense population	203 mm	132 mm	n/a	n/a	1660 g	C	0	n/a	
321	MF# 5602	no markings, dense population	215 mm	131 mm	n/a	n/a	1720 g	C	0	n/a	
322	MF# 5603	no markings, dense population	245 mm	156 mm	n/a	n/a	2440 g	C	0	n/a	
323	MF# 5604	no markings, dense population	221 mm	135 mm	n/a	n/a	1900 g	C	0	n/a	
324	MF# 5605	no markings, dense population	219 mm	137 mm	n/a	n/a	1840 g	C	0	n/a	
325	MF# 5606	no markings, dense population	211 mm	133 mm	n/a	n/a	1900 g	C	0	n/a	
326	MF# 5607	no markings, dense population	239 mm	139 mm	n/a	n/a	2400 g	C	0	n/a	
327	MF# 5608	no markings, dense population	203 mm	128 mm	n/a	n/a	1420 g	C	0	n/a	
328	MF# 5609	no markings, dense population	169 mm	98 mm	n/a	n/a	880 g	C	0	n/a	
329	MF# 5610	no markings, dense population	184 mm	118 mm	n/a	n/a	1180 g	C	0	n/a	
330	MF# 5611	no markings, dense population	153 mm	88 mm	n/a	n/a	740 g	C	0	n/a	
331	MF# 5612	no markings, dense population	192 mm	118 mm	n/a	n/a	1580 g	C	0	n/a	
332	MF# 5613	no markings, dense population	209 mm	131 mm	n/a	n/a	1620 g	C	0	n/a	
333	MF# 5614	no markings, dense population	207 mm	125 mm	n/a	n/a	1380 g	C	0	n/a	
334	MF# 5615	no markings, dense population	209 mm	123 mm	n/a	n/a	1880 g	C	0	n/a	
335	MF# 5616	no markings, dense population	220 mm	138 mm	n/a	n/a	2000 g	C	0	n/a	
336	MF# 5617	no markings, dense population	197 mm	120 mm	n/a	n/a	1440 g	C	0	n/a	
337	MF# 5618	no markings, dense population	214 mm	140 mm	n/a	n/a	2002 g	C	0	n/a	
338	MF# 5619	no markings, dense population	208 mm	127 mm	n/a	n/a	1800 g	C	0	n/a	
339	MF# 5620	no markings, dense population	226 mm	134 mm	n/a	n/a	2200 g	C	0	n/a	
340	MF# 5621	no markings, dense population	209 mm	126 mm	n/a	n/a	1700 g	C	0	n/a	
341	MF# 5622	no markings, dense population	228 mm	139 mm	n/a	n/a	2220 g	C	0	n/a	
342	MF# 5623	no markings, dense population	226 mm	127 mm	n/a	n/a	2020 g	C	0	n/a	
343	MF# 5624	no markings, dense population	177 mm	112 mm	n/a	n/a	1080 g	C	0	n/a	
344	MF# 5625	no markings, dense population	203 mm	136 mm	n/a	n/a	1680 g	C	0	n/a	
345	MF# 5626	no markings, dense population	199 mm	126 mm	n/a	n/a	1840 g	C	0	n/a	
346	MF# 5627	no markings, dense population	191 mm	120 mm	n/a	n/a	1400 g	C	0	n/a	
347	MF# 5628	no markings, dense population	196 mm	126 mm	n/a	n/a	1460 g	C	0	n/a	
348	MF# 5629	no markings, dense population	214 mm	136 mm	n/a	n/a	2040 g	C	0	n/a	
349	MF# 5630	no markings, dense population	253 mm	150 mm	n/a	n/a	2880 g	C	0	n/a	
350	MF# 5631	no markings, dense population	191 mm	123 mm	n/a	n/a	1480 g	C	0	n/a	
351	MF# 5632	no markings, dense population	193 mm	122 mm	n/a	n/a	1420 g	C	0	n/a	
352	MF# 5633	no markings, dense population	198 mm	140 mm	n/a	n/a	1660 g	C	0	n/a	
353	MF# 5634	no markings, dense population	220 mm	138 mm	n/a	n/a	1720 g	C	0	n/a	
354	MF# 5635	no markings, dense population	242 mm	150 mm	n/a	n/a	3180 g	C	0	n/a	
355	MF# 5636	no markings, dense population	223 mm	136 mm	n/a	n/a	2080 g	C	0	n/a	

	A	B	C	D	E	F	G	H	I	J	K	L
1	MF #	Collector #	Family	*Genus*	*Species*	*Subspecies*	Sex	Specific Locality	County	State	Country	Date Collected
356	MF# 5637	GLL # 281	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
357	MF# 5638	GLL # 282	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
358	MF# 5639	GLL # 283	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
359	MF# 5640	GLL # 284	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
360	MF# 5641	GLL # 285	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
361	MF# 5642	GLL # 286	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
362	MF# 5643	GLL # 287	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
363	MF# 5644	GLL # 288	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
364	MF# 5645	GLL # 289	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
365	MF# 5646	GLL # 290	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
366	MF# 5647	GLL # 291	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
367	MF# 5648	GLL # 292	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
368	MF# 5649	GLL # 293	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
369	MF# 5650	GLL # 294	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
370	MF# 5651	GLL # 295	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
371	MF# 5652	GLL # 296	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
372	MF# 5653	GLL # 297	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
373	MF# 5654	GLL # 298	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
374	MF# 5655	GLL # 299	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
375	MF# 5656	GLL # 300	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
376	MF# 5657	GLL # 301	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
377	MF# 5658	GLL # 302	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
378	MF# 5659	GLL # 303	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
379	MF# 5660	GLL # 304	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
380	MF# 5661	GLL # 305	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
381	MF# 5662	GLL # 306	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
382	MF# 5663	GLL # 307	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
383	MF# 5664	GLL # 308	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
384	MF# 5665	GLL # 309	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
385	MF# 5666	GLL # 310	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
386	MF# 5667	GLL # 311	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
387	MF# 5668	GLL # 312	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
388	MF# 5669	GLL # 313	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
389	MF# 5670	GLL # 314	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	03/15/02
390	MF# 5795	GLL #316	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA	USA	04/03/06
391	MF# 5994	GL # 216	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Concordia Turtle Farm, Wildsville, LA		LA		03/15/02
392	MF# 5470	GLL #25	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	01/29/02
393	MF# 5805	GLL #326	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
394	MF# 5806	GLL #327	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
395	MF# 5807	GLL #328	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
396	MF# 5808	GLL #329	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/02/02
397	MF# 5809	GLL #330	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/04/02
398	MF# 5861	GLL #382	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
399	MF# 5862	GLL #383	Emydinae	*Trachemys*	*scripta*	*elegans*	U	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
400	MF# 5863	GLL #384	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
401	MF# 5864	GLL #385	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
402	MF# 5865	GLL #386	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
403	MF# 5867	GLL #388	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Cypress Point, Aquarena Springs, San Marcos, Texas (basking trap)	Hays	TX	USA	04/09/02
404	MF# 5890	GLL #411	Emydinae	*Trachemys*	*scripta*	*elegans*	U	Deanville (mom's lake)		TX	USA	05/11/02
405	MF# 5891	GLL #412	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Deanville (mom's lake)		TX	USA	05/11/02
406	MF# 5892	GLL #413	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Deanville (mom's lake)		TX	USA	05/11/02
407	MF# 5893	GLL #414	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
408	MF# 5894	GLL #415	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
409	MF# 5895	GLL #416	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
410	MF# 5896	GLL #417	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Deanville (mom's lake)		TX	USA	05/12/02
411	MF# 5897	GLL #418	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Deanville (mom's lake)		TX	USA	05/12/02
412	MF# 5898	GLL #419	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
413	MF# 5899	GLL #420	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Deanville (mom's lake)		TX	USA	05/12/02
414	MF# 5900	GLL #421	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02

	A	M	N	O	P	Q	R	S	T	U	V
1	MF #	Additional Data	PlastronLength/SVL	PlastronWidth/TL	CarapaceLength	CarapaceWidth	Wgt	W/C/WCCR	% parasitemia	parasite mmts (microns)	PCR test
356	MF# 5637	no markings, dense population	223 mm	141 mm	n/a	n/a	2140 g	C	0	n/a	
357	MF# 5638	no markings, dense population	216 mm	132 mm	n/a	n/a	1780 g	C	0	n/a	
358	MF# 5639	no markings, dense population	224 mm	139 mm	n/a	n/a	1960 g	C	0	n/a	
359	MF# 5640	no markings, dense population	239 mm	151 mm	n/a	n/a	2700 g	C	0	n/a	
360	MF# 5641	no markings, dense population	203 mm	118 mm	n/a	n/a	1420 g	C	0	n/a	
361	MF# 5642	no markings, dense population	228 mm	133 mm	n/a	n/a	1860 g	C	0	n/a	
362	MF# 5643	no markings, dense population	199 mm	131 mm	n/a	n/a	1680 g	C	0	n/a	
363	MF# 5644	no markings, dense population	213 mm	131 mm	n/a	n/a	1880 g	C	0	n/a	
364	MF# 5645	no markings, dense population	221 mm	136 mm	n/a	n/a	1880 g	C	0	n/a	
365	MF# 5646	no markings, dense population	212 mm	140 mm	n/a	n/a	2020 g	C	0	n/a	
366	MF# 5647	no markings, dense population	208 mm	136 mm	n/a	n/a	1620 g	C	0	n/a	
367	MF# 5648	no markings, dense population	217 mm	141 mm	n/a	n/a	2100 g	C	0	n/a	
368	MF# 5649	no markings, dense population	217 mm	135 mm	n/a	n/a	1840 g	C	0	n/a	
369	MF# 5650	no markings, dense population	214 mm	132 mm	n/a	n/a	1700 g	C	0	n/a	
370	MF# 5651	no markings, dense population	200 mm	125 mm	n/a	n/a	1400 g	C	0	n/a	
371	MF# 5652	no markings, dense population	111 mm	76 mm	n/a	n/a	240 g	C	0	n/a	
372	MF# 5653	no markings, dense population	119 mm	85 mm	n/a	n/a	320 g	C	0	n/a	
373	MF# 5654	no markings, dense population	130 mm	84 mm	n/a	n/a	360 g	C	0	n/a	
374	MF# 5655	no markings, dense population	117 mm	80 mm	n/a	n/a	300 g	C	0	n/a	
375	MF# 5656	no markings, dense population	123 mm	86 mm	n/a	n/a	340 g	C	0	n/a	
376	MF# 5657	no markings, dense population	121 mm	82 mm	n/a	n/a	n/a	C	0	n/a	
377	MF# 5658	no markings, dense population	125 mm	82 mm	n/a	n/a	340 g	C	0	n/a	
378	MF# 5659	no markings, dense population	102 mm	72 mm	n/a	n/a	220 g	C	0	n/a	
379	MF# 5660	no markings, dense population	120 mm	82 mm	n/a	n/a	320 g	C	0	n/a	
380	MF# 5661	no markings, dense population	106 mm	78 mm	n/a	n/a	240 g	C	0	n/a	
381	MF# 5662	no markings, dense population	99 mm	75 mm	n/a	n/a	160 g	C	0	n/a	
382	MF# 5663	no markings, dense population	111 mm	83 mm	n/a	n/a	280 g	C	0	n/a	
383	MF# 5664	no markings, dense population	110 mm	79 mm	n/a	n/a	240 g	C	0	n/a	
384	MF# 5665	no markings, dense population	87 mm	65 mm	n/a	n/a	140 g	C	0	n/a	
385	MF# 5666	no markings, dense population	102 mm	75 mm	n/a	n/a	200 g	C	0	n/a	
386	MF# 5667	no markings, dense population	98 mm	70 mm	n/a	n/a	160 g	C	0	n/a	
387	MF# 5668	no markings, dense population	65 mm	52 mm	n/a	n/a	60 g	C	0	n/a	
388	MF# 5669	no markings, dense population	93 mm	70 mm	n/a	n/a	160 g	C	0	n/a	
389	MF# 5670	no markings, dense population	80 mm	62 mm	n/a	n/a	120 g	C	0	n/a	
390	MF# 5795	no markings	650 mm	520 mm	n/a	n/a	60 g	C	0	n/a	
391	MF# 5994		214 mm	136 mm	n/a	n/a	1820 g	W	0	n/a	
392	MF# 5470	LM1,2 RM3 LG3	143 mm	n/a	145 mm	120 mm	550 g	W	0 2	14 x 7	
393	MF# 5805	LM1,2 RM10 LG2, pit#031-588-524	198 mm	n/a	213 mm	161 mm	1450 g	W	0 86	14 x 6	pos
394	MF# 5806	LM1,3 RM10 RH1	n/a	n/a	n/a	n/a	n/a	W	0 21	12 x 4	
395	MF# 5807	LM1,2 RM9 LG2, pit#031-595-001	188 mm	n/a	208 mm	158 mm	1400 g	W	2	14 x 6	pos
396	MF# 5808	LM1,9 RM4 RH1	n/a	n/a	n/a	n/a	n/a	W	0 03	12 x 3	
397	MF# 5809	LM1,2 RM11 pit#031-576-269	183 mm	n/a	202 mm	152 mm	1250 g	W	0	n/a	neg
398	MF# 5861	LM1,2 RM12 LG2, pit#031-598-604	181 mm	n/a	192 mm	139 mm	1000 g	W	0 29	14 x 5	pos
399	MF# 5862	LM2,12 RM6 RA, pit#031-584-527	180 mm	n/a	197 mm	155 mm	1200 g	W	0 12	12-14 x 4	
400	MF# 5863	LM1,2 RM9 RH	n/a	n/a	n/a	n/a	n/a	W	0 1	13 x 6	
401	MF# 5864	LM1,3 RM7 RH, pit#024-823-304	185 mm	n/a	207 mm	158 mm	1200 g	W	1 67	14 x 5	pos
402	MF# 5865	LM6 RM12 LH, pit#012-572-630	204 mm	n/a	219 mm	169 mm	1700 g	W	0 65	14 x 4	pos
403	MF# 5867	LM1,2 RM5 LG2	123 mm	n/a	139 mm	107 mm	375 g	W	0 2	12 x 4	
404	MF# 5890	TSE1 (drilled #1 according to IUCN field form)	7 7 in	5 5 in	8 5 in	6 0 in	2 25 lbs	W	0 22	15 x 7	
405	MF# 5891	TSE2	7 25 in	5 0 in	7 25 in	6 0 in	1 75 lbs	W	0 01	n/a	
406	MF# 5892	TSE3	8 5 in	5 5 in	9 0 in	7 0 in	3 0 lbs	W	0 56	16 x 3	pos
407	MF# 5893	TSE4	7 0 in	4 5 in	7 5 in	5 5 in	2 0 lbs	W	0 38	13 x 4	pos
408	MF# 5894	TSE5	5 75 in	4 0 in	6 0 in	5 0 in	1 0 lbs	W	0 22	14 x 5	neg
409	MF# 5895	TSE6	6 5 in	4 25 in	7 5 in	5 5 in	2 0 lbs	W	0 08	12 x 5	
410	MF# 5896	TSE7	8 0 in	5 25 in	8 25 in	6 5 in	3 0 lbs	W	0 02	16 x 5	
411	MF# 5897	TSE8	8 0 in	5 25 in	8 25 in	6 25 in	2 8 lbs	W	0 05	14 x 4	
412	MF# 5898	TSE9	6 25 in	4 0 in	6 5 in	5 25 in	1 0 lbs	W	1 88	16 x 5	pos
413	MF# 5899	TSE10	8 5 in	5 75 in	9 0 in	7 0 in	4 0 lbs	W	0 74	9 x 4	
414	MF# 5900	TSE11	6 25 in	4 5 in	7 0 in	5 5 in	1 75 lbs	W	0 01	12 x 3	

	A	B	C	D	E	F	G	H	I	J	K	L
1	MF #	Collector #	Family	*Genus*	*Species*	*Subspecies*	Sex	Specific Locality	County	State	Country	Date Collected
415	MF# 5901	GLL #422	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
416	MF# 5902	GLL #423	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Deanville (mom's lake)		TX	USA	05/12/02
417	MF# 5903	GLL #424	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
418	MF# 5904	GLL #425	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
419	MF# 5905	GLL #426	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
420	MF# 5906	GLL #427	Emydinae	*Trachemys*	*scripta*	*elegans*	U	Deanville (mom's lake)		TX	USA	05/12/02
421	MF# 5907	GLL #428	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
422	MF# 5908	GLL #429	Emydinae	*Trachemys*	*scripta*	*elegans*	U	Deanville (mom's lake)		TX	USA	05/12/02
423	MF# 5909	GLL #430	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
424	MF# 5910	GLL #431	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
425	MF# 5911	GLL #432	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Deanville (mom's lake)		TX	USA	05/12/02
426	MF# 5912	GLL #433	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Deanville (mom's lake)		TX	USA	05/12/02
427	MF#7378	GL# 434	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Griffith League Ranch		TX	USA	06/12/02
428	MF# 5872	GLL #393	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Oasis Ranch, West Texas		TX	USA	04/12/02
429	MF# 5873	GLL #394	Emydinae	*Trachemys*	*scripta*	*elegans*	M	Oasis Ranch, West Texas		TX	USA	04/12/02
430	MF# 5880	GLL #401	Emydinae	*Trachemys*	*scripta*	*elegans*	F	Oasis Ranch, West Texas		TX	USA	04/13/02
431	MF# 5874	GLL #395	Trionychidae	*Trionyx (Apalone)*	*spinifera*		M	Oasis Ranch, West Texas		TX	USA	04/13/02
432												
433												
434												
435												

	A	M	N	O	P	Q	R	S	T	U	V
1	MF #	Additional Data	PlastronLength/SVL	PlastronWidth/TL	CarapaceLength	CarapaceWidth	Wgt	W/C/WCCR	% parasitemia	parasite mmts (microns)	PCR test
415	MF# 5901	TSE12	8 0 in	4 75 in	8 0 in	6 25 in	2 8 lbs	W	0	n/a	neg
416	MF# 5902	TSE13	8 25 in	5 5 in	8 5 in	6 5 in	3 0 lbs	W	0 6	12 x 4	
417	MF# 5903	TSE14	6 25 in	4 25 in	6 75 in	5 25 in	1 5 lbs	W	0 18	12 x 4	
418	MF# 5904	TSE15	6 5 in	4 25 in	7 25 in	5 25 in	1 5 lbs	W	0 46	16 x 5	
419	MF# 5905	TSE16	6 25 in	4 5 in	6 5 in	5 5 in	1 5 lbs	W	0 05	8 x 4	
420	MF# 5906	TSE17	5 5 in	5 25 in	6 0 in	5 0 in	1 0 lb	W	0	n/a	neg
421	MF# 5907	TSE18	7 0 in	4 75 in	7 5 in	6 75 in	2 0 lb	W	0 1	6-12 x 4	
422	MF# 5908	TSE19	4 5 in	3 5 in	5 0 in	4 0 in	0 5 lb	W	0	n/a	
423	MF# 5909	TSE20	5 0 in	3 75 in	6 5 in	4 5 in	0 5 lb	W	0	n/a	
424	MF# 5910	TSE21	6 25 in	4 25 in	6 25 in	5 25 in	1 0 lb	W	0	n/a	
425	MF# 5911	TSE22	7 5 in	5 0 in	8 0 in	6 0 in	2 5 lb	W	0	n/a	
426	MF# 5912	TSE23	6 75 in	4 5 in	7 5 in	5 5 in	2 0 lb	W	0 89	16 x 5	
427	MF#7378		197 mm	130 mm	n/a	n/a	n/a	W	0 08	8 x 4	
428	MF# 5872	TSE1 (drilled #1 according to IUCN field form)	150 mm	100 mm	172 mm	125 mm	660 g	W	0	n/a	neg
429	MF# 5873	TSE2 (drilled #2 according to IUCN field form)	180 mm	110 mm	240 mm	170 mm	1040 g	W	0	n/a	
430	MF# 5880	notched TSE3	120 mm	100 mm	130 mm	120 mm	8 g ??	W	0	n/a	neg
431	MF# 5874		130 mm	141 mm	186 mm	142 mm	760 g	W	0	n/a	neg
432											
433											
434											
435											

APPENDIX B

CHEMICALS

Blood Storage Buffer
=250ml volume
3.03g TRIS
9.31 g EDTA Na_2
2.5 g SDS
fill to 250 ml volume with ddH_2O

Buffer A (for PCR)
=250 ml volume
9.0825 g TRIS
0.508 g $MgCl_2$ $6H_2O$
2.477g $(NH_4)2SO_4$
pH adjusted to 8.5

APPENDIX C

ALIGNMENT OF 18S SEQUENCES FOR APICOMPLEXANS

Appendix C


AF173605-Alligator.mississi...
M59392-Heterodon.platyrhinos
M59400-Sceloporus.undulatus
M59398-Trachemys.scripta
U16369-Babesia.odocoilei
U16370-Babesia.divergens
AF060976-Caryospora.bigen...
AF060975-Caryospora.bigen...
AY078092-Colpodella.pontica
AY142075-Colpodella.sp.
AF112573-Cryptosporidium....
AF112575-Crypto.felis
AF112574-Crypto.meleagridis
AF112576-Crypto.parvum
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AF115377-Crypto.parvum
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AF161858-Crypto.parvum
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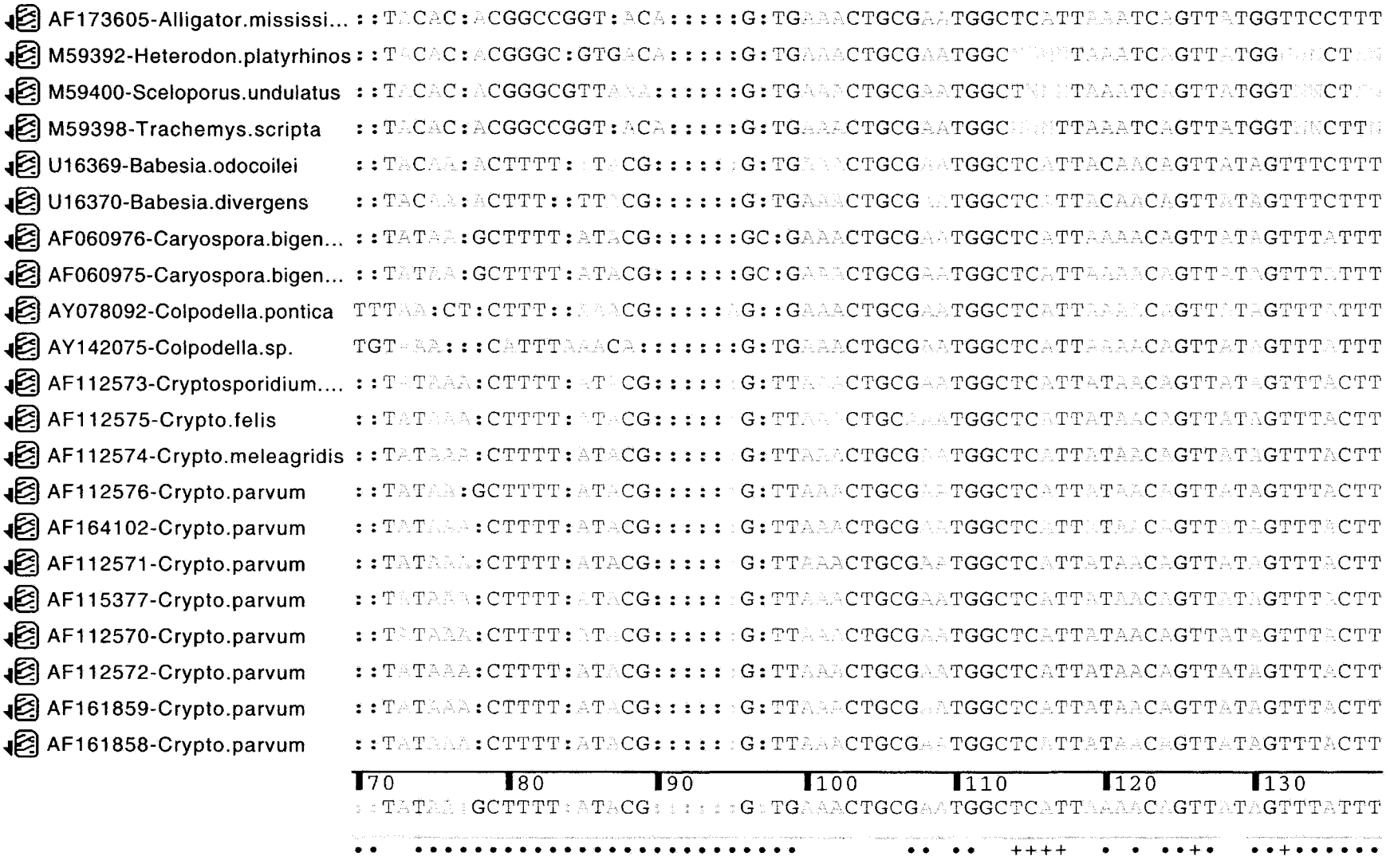
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U16370-Babesia.divergens
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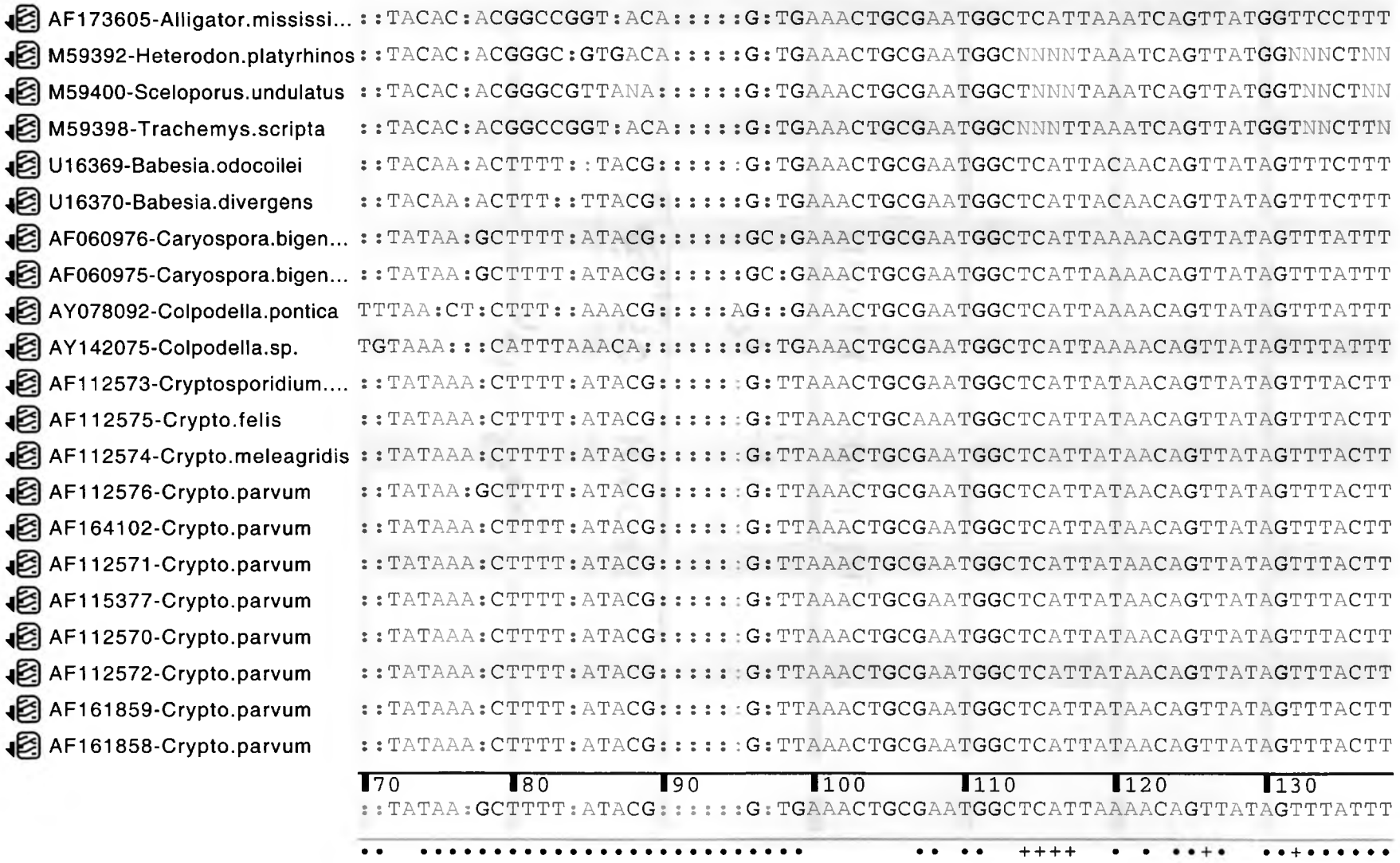
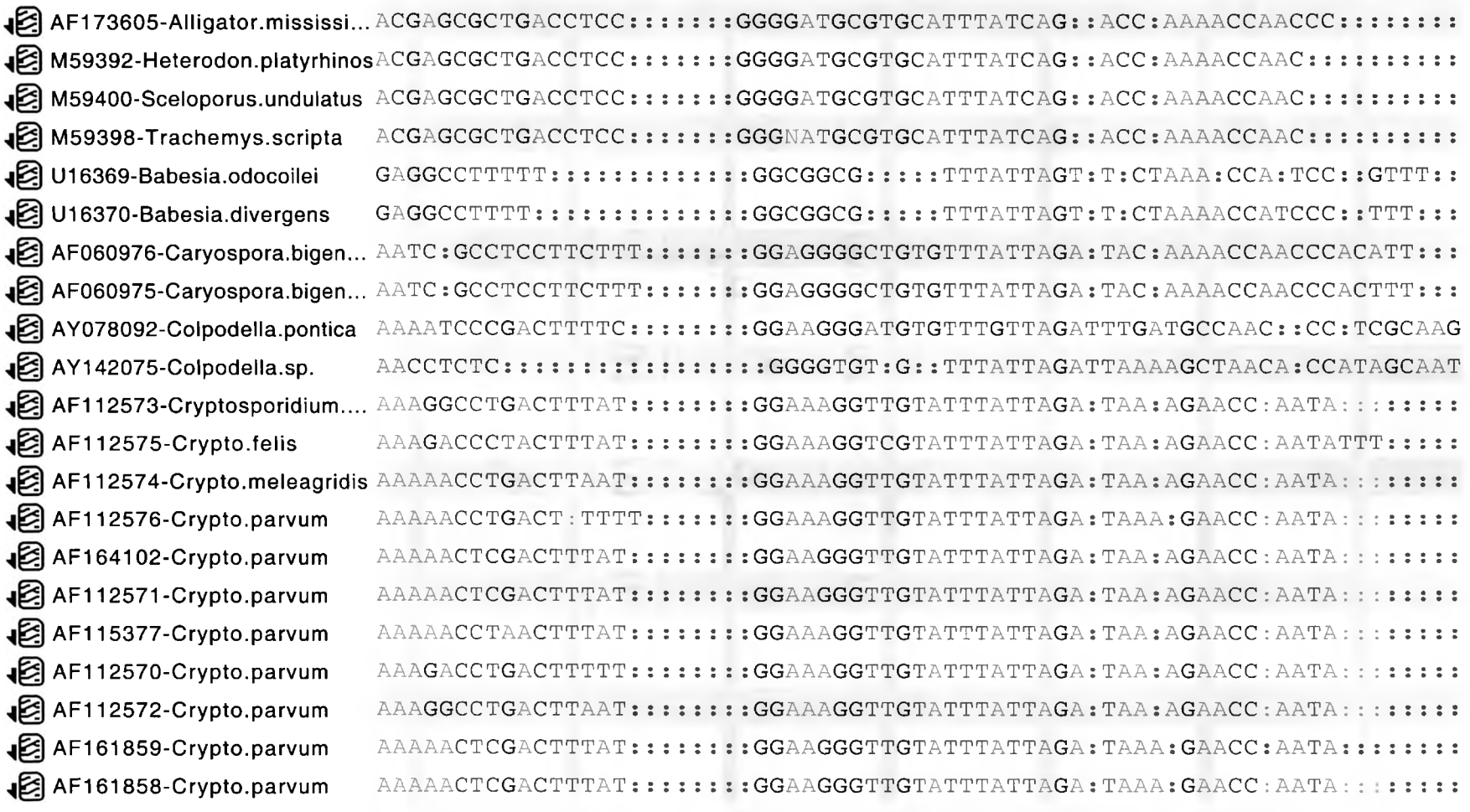


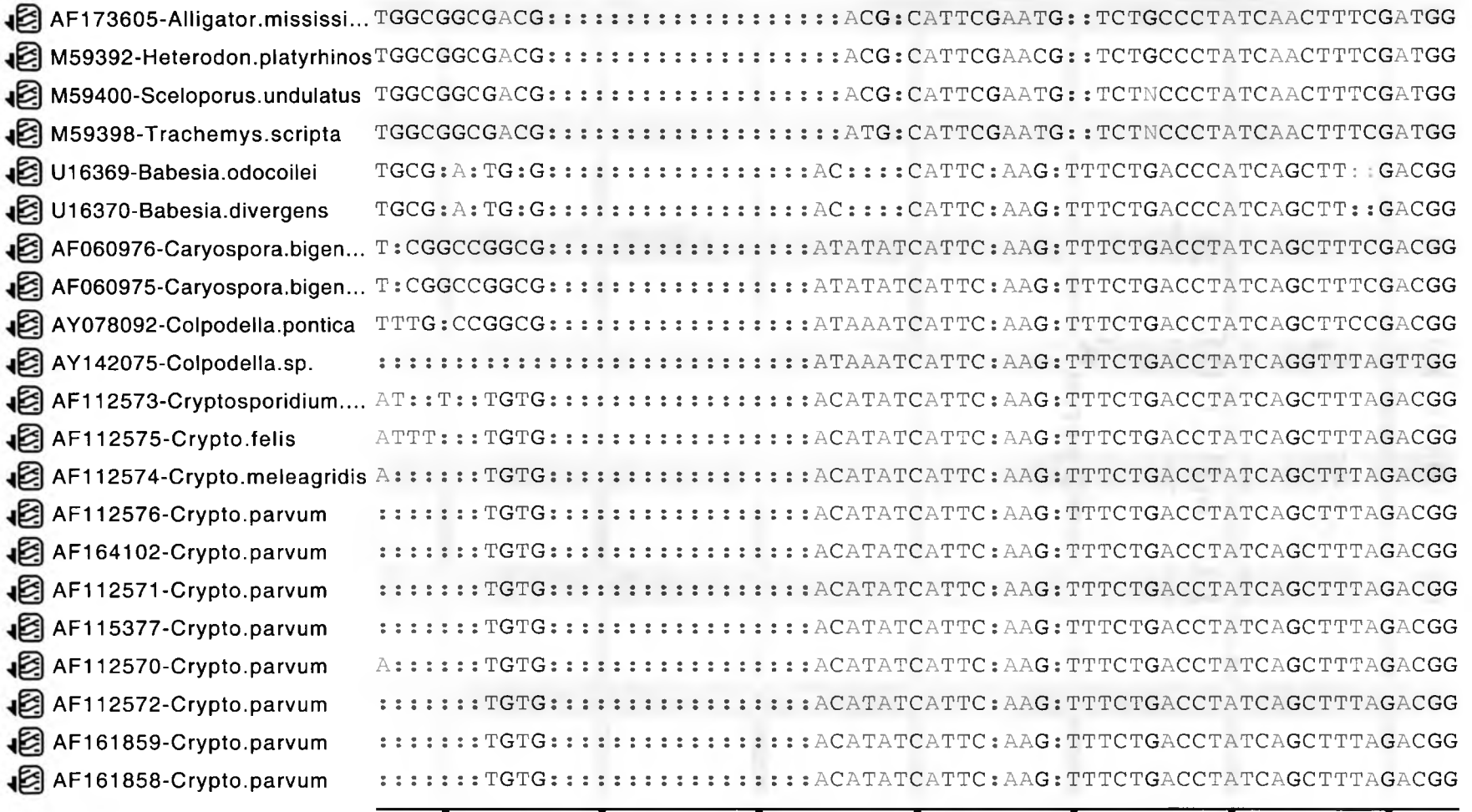
Appendix C (cont.)

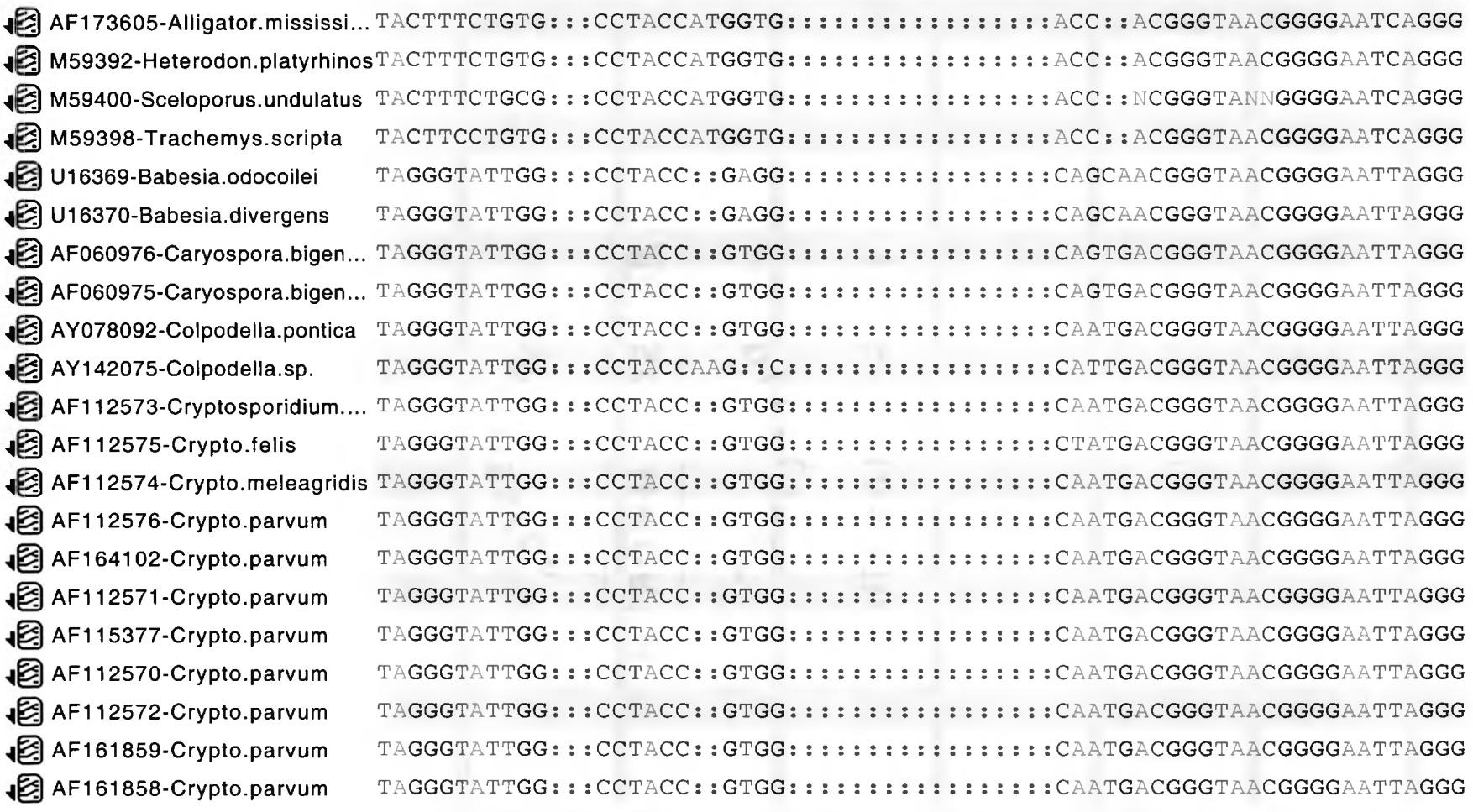
Appendix C (cont.)



Appendix C (cont.)

Appendix C (cont.)

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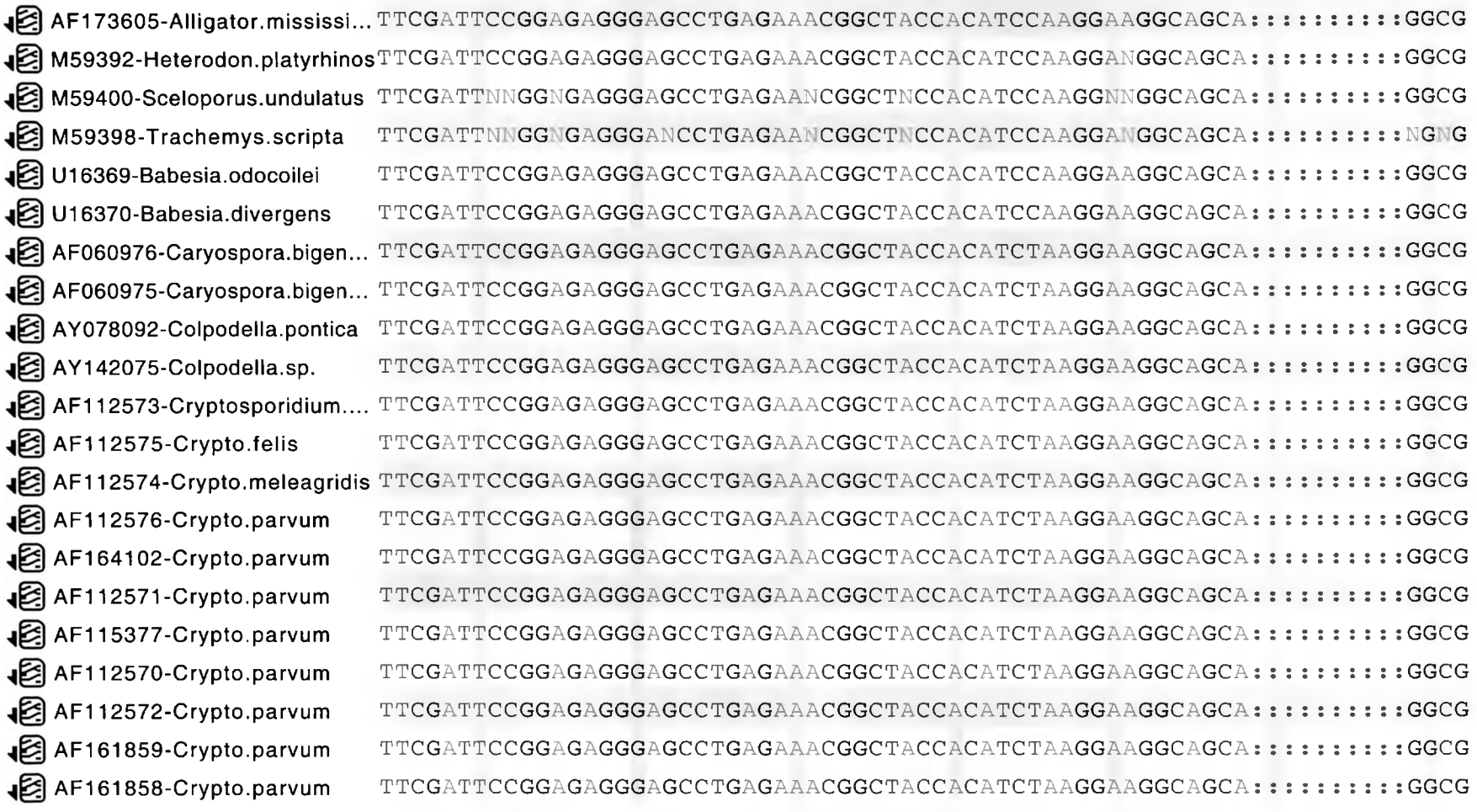
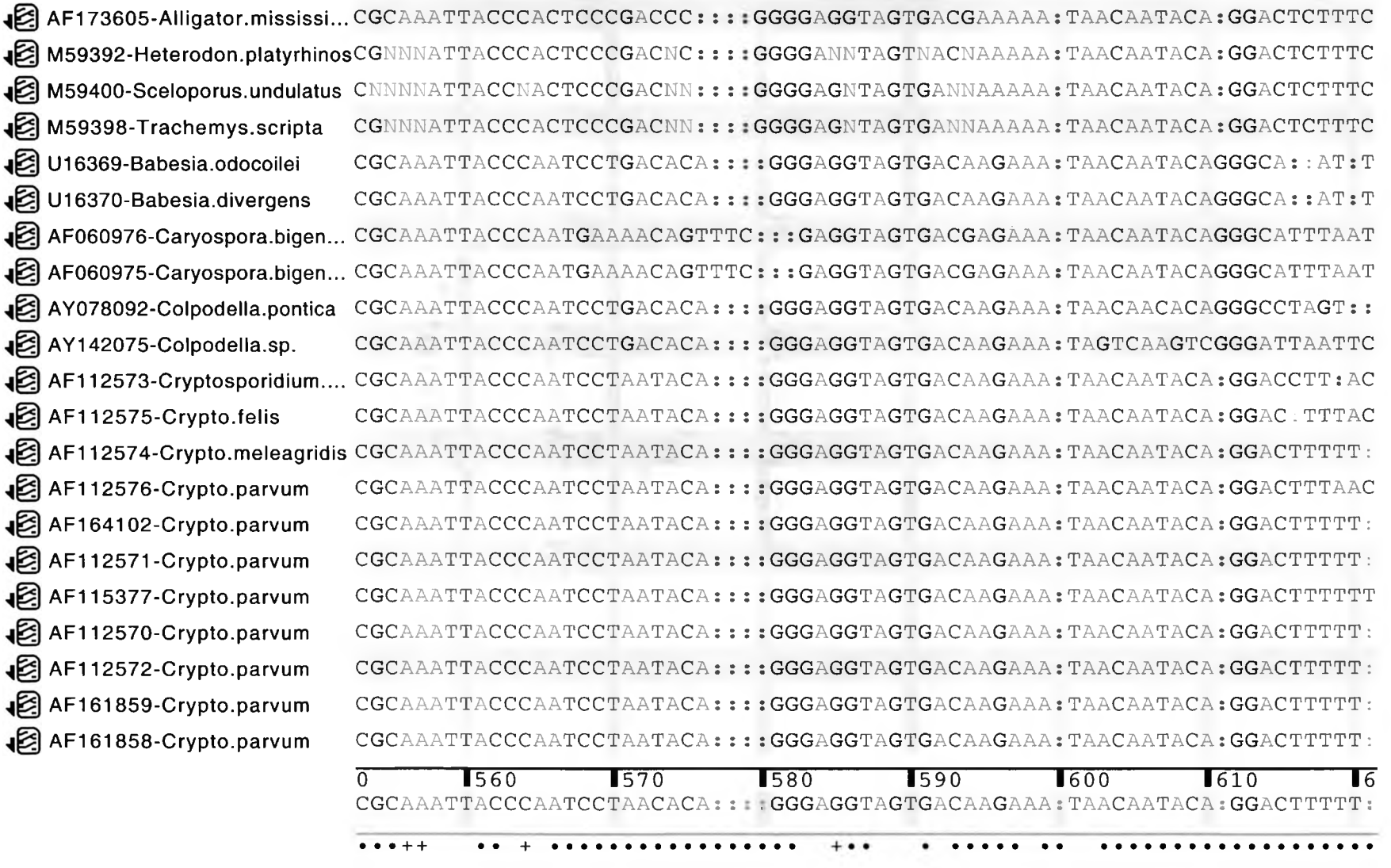
Appendix C (cont.)

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AY142075-Colpodella.sp. CGCAAATTACCCAATCCTGACACA::::GGGAGGTAGTGACAAGAAA:TAGTCAAGTCGGGATTAATTC
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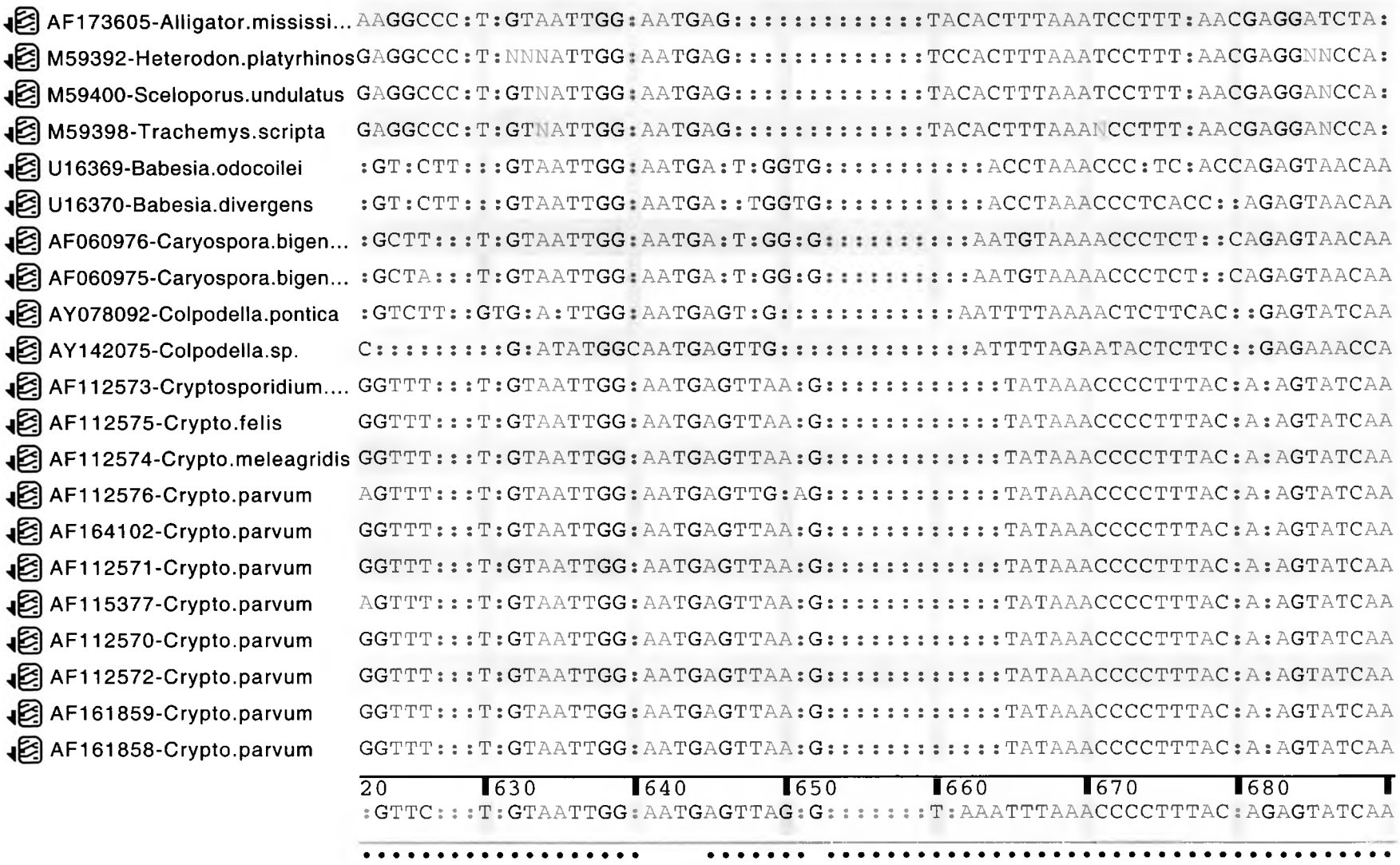
Appendix C (cont.)



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U16369-Babesia.odocoilei        TTGGAGGGCAAGTCTGGTGCCAGCAGCCGC:GGTAATTCCAGCTCCAATAGCGTATATTAAACTTGTTG
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AY078092-Colpodella.pontica     TTGGAGGGCAAGTCTGGTGCCAGCAGCCGC:GGTAATTCCAGCTCCAATAGCGTGTATTAAAGTTGTTG
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Appendix C (cont.)

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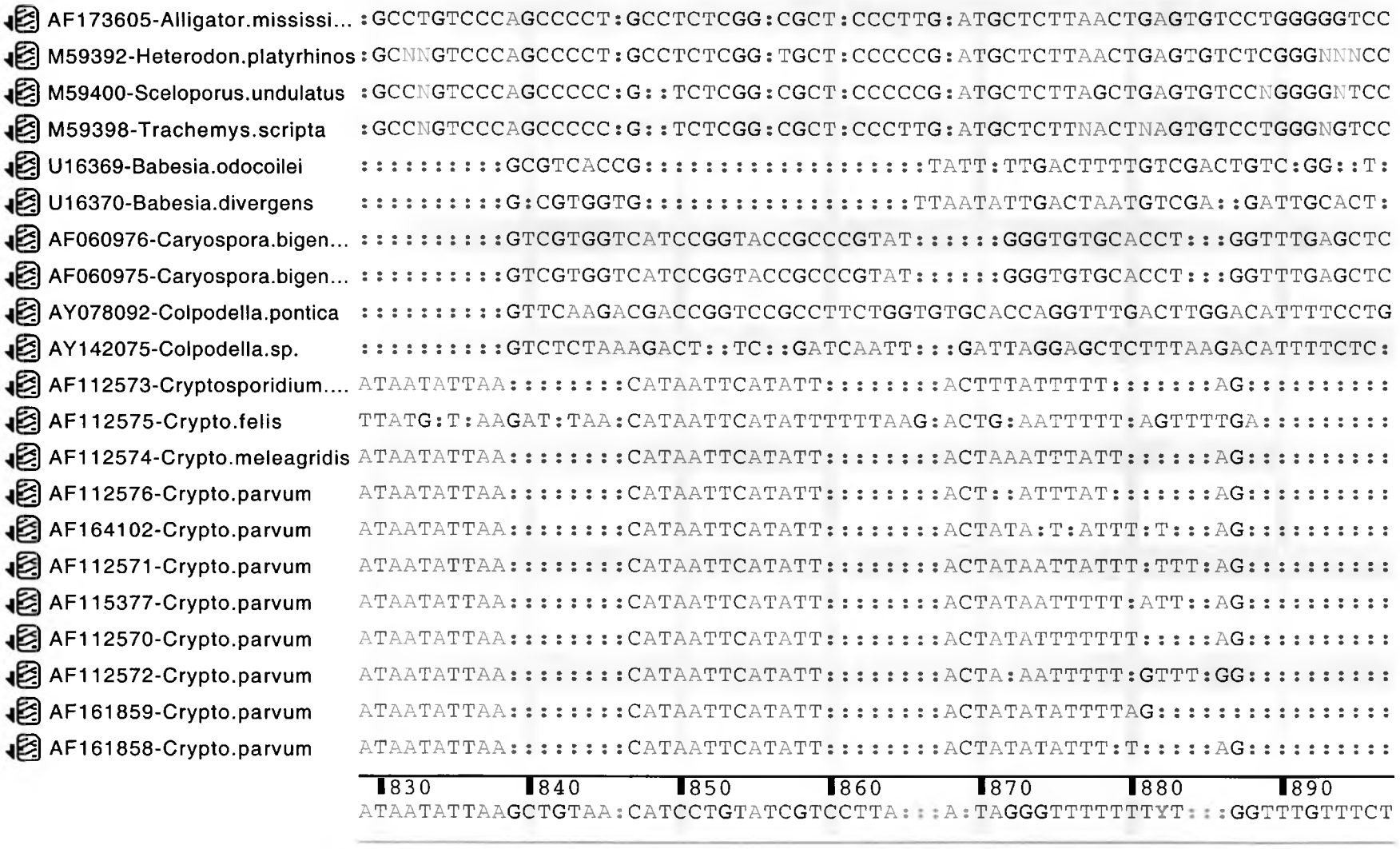
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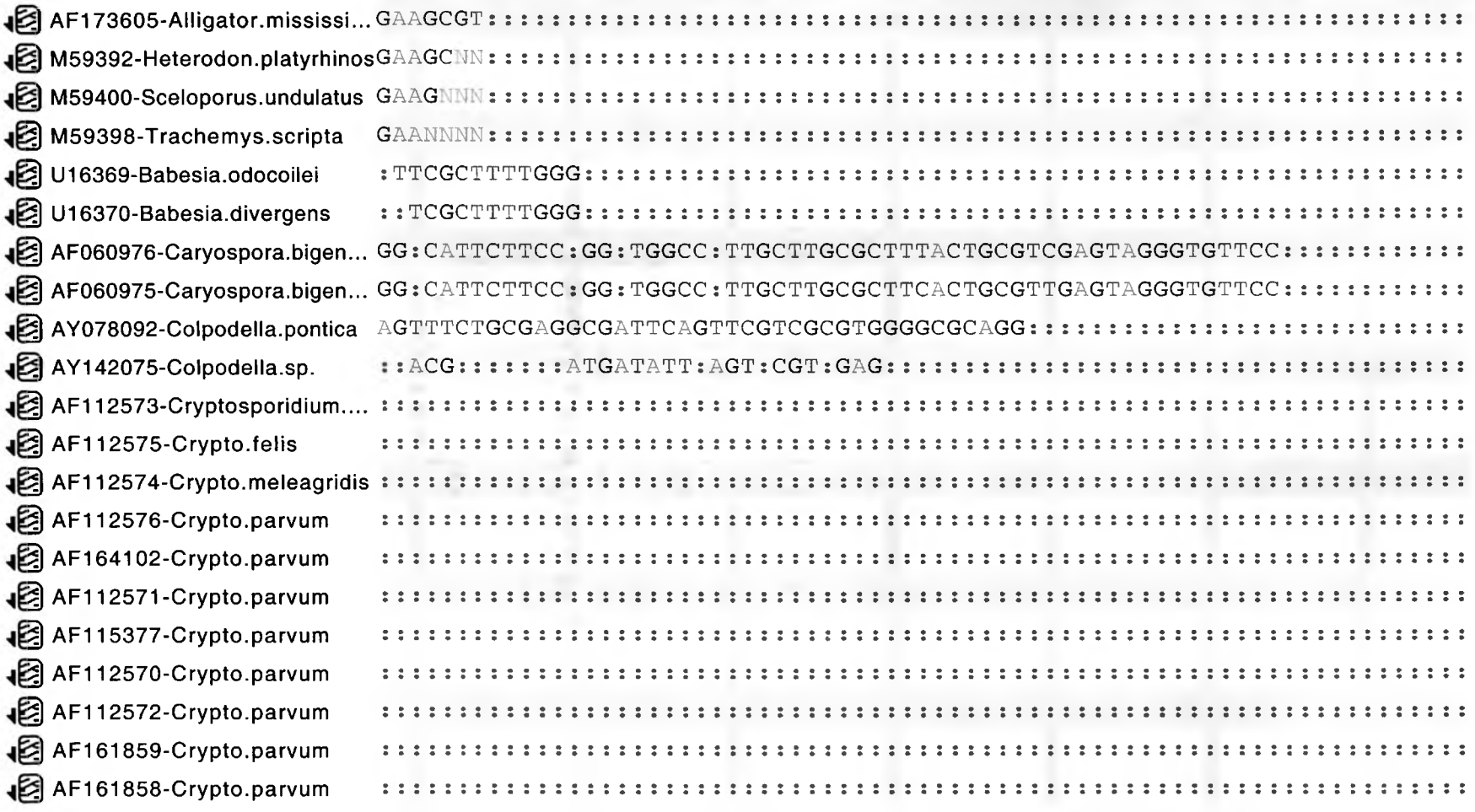
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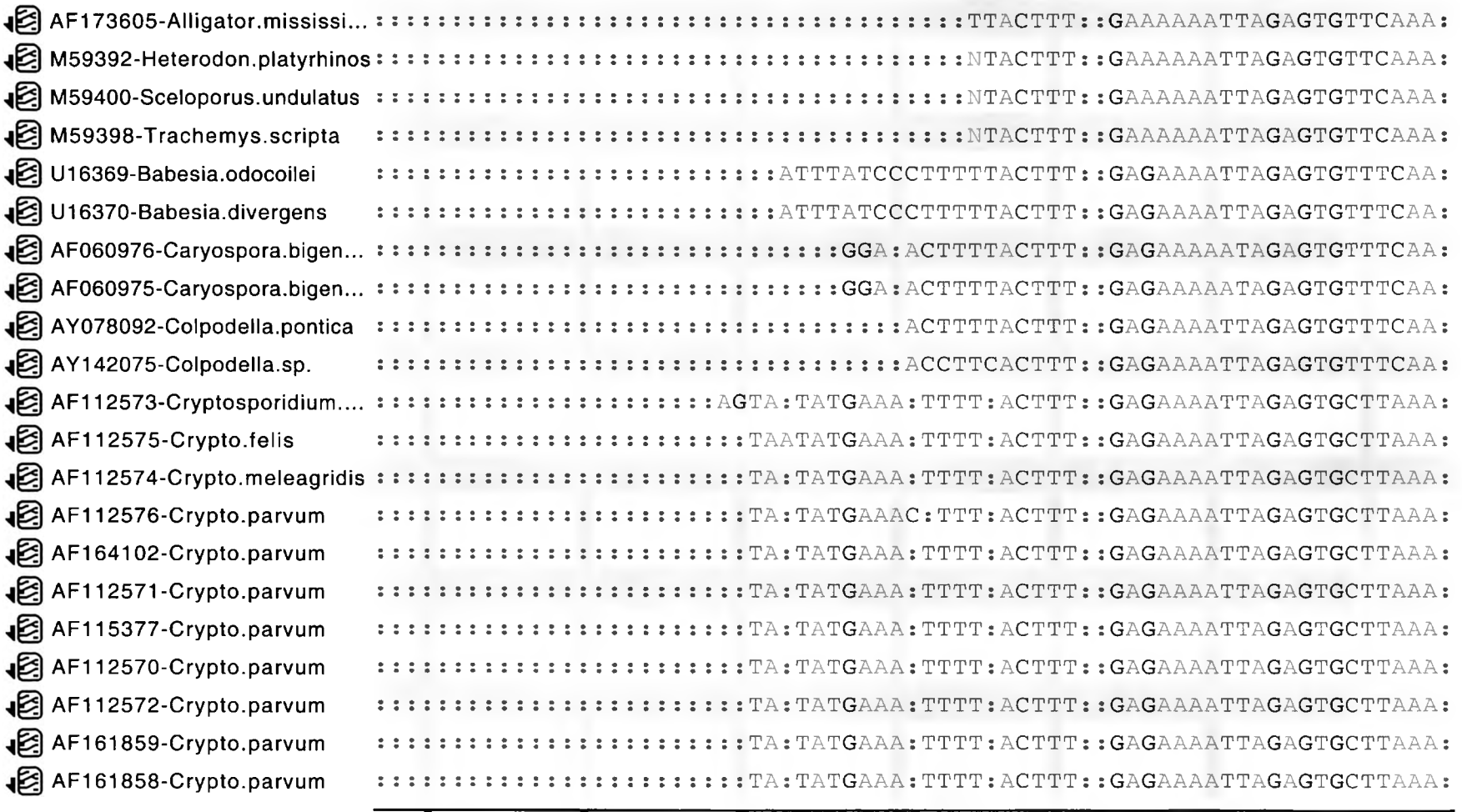
Appendix C (cont.)



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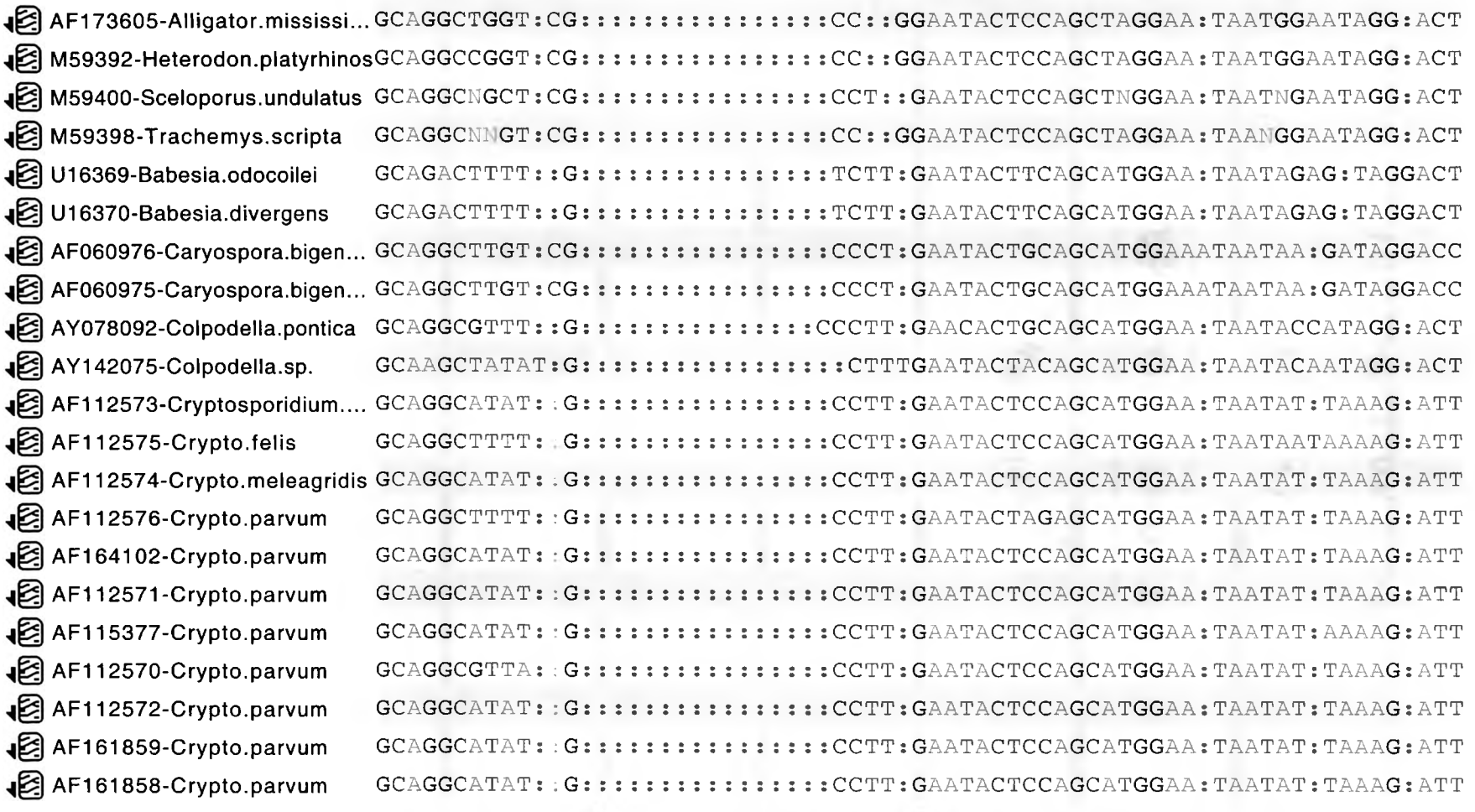

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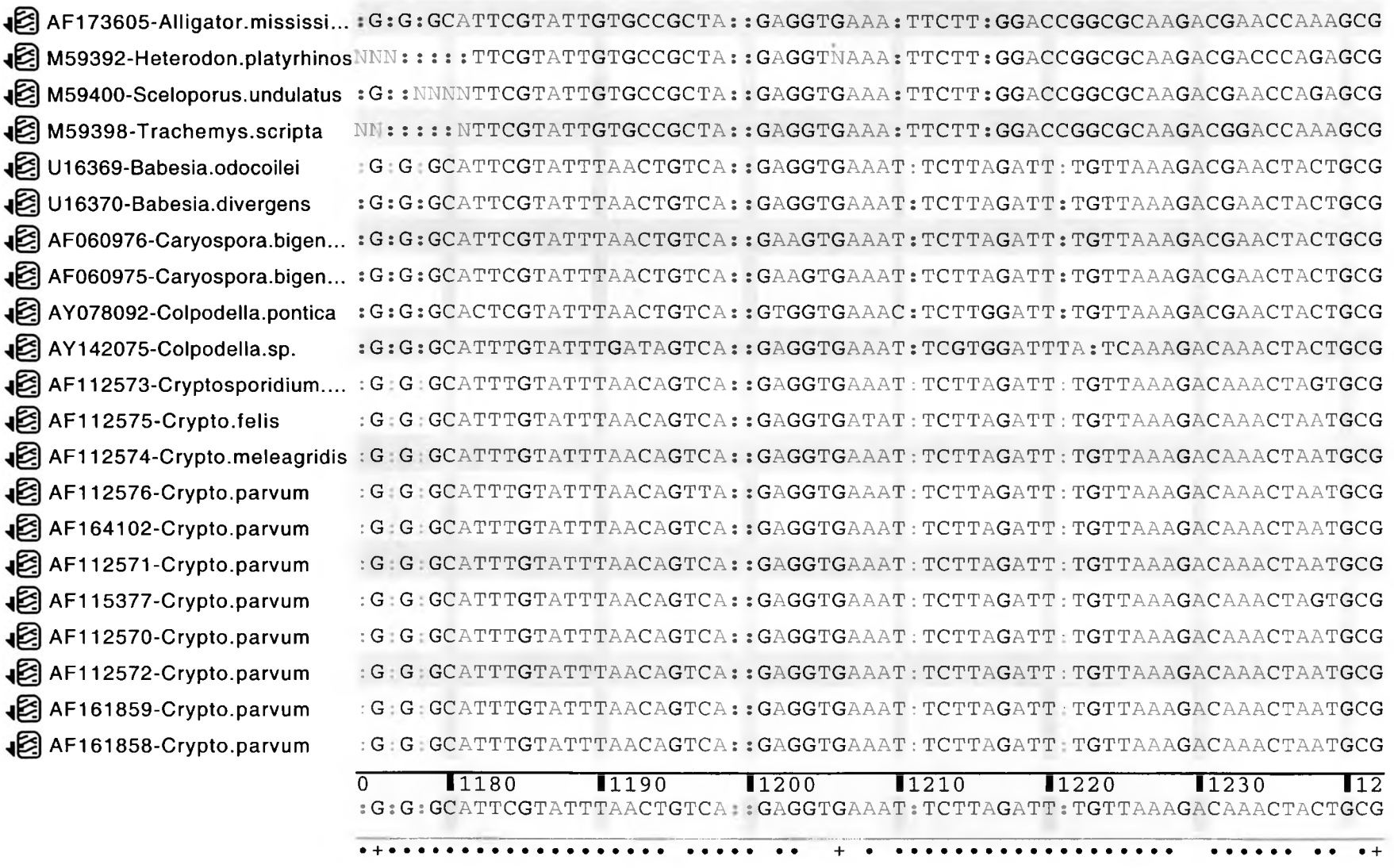
Appendix C (cont.)



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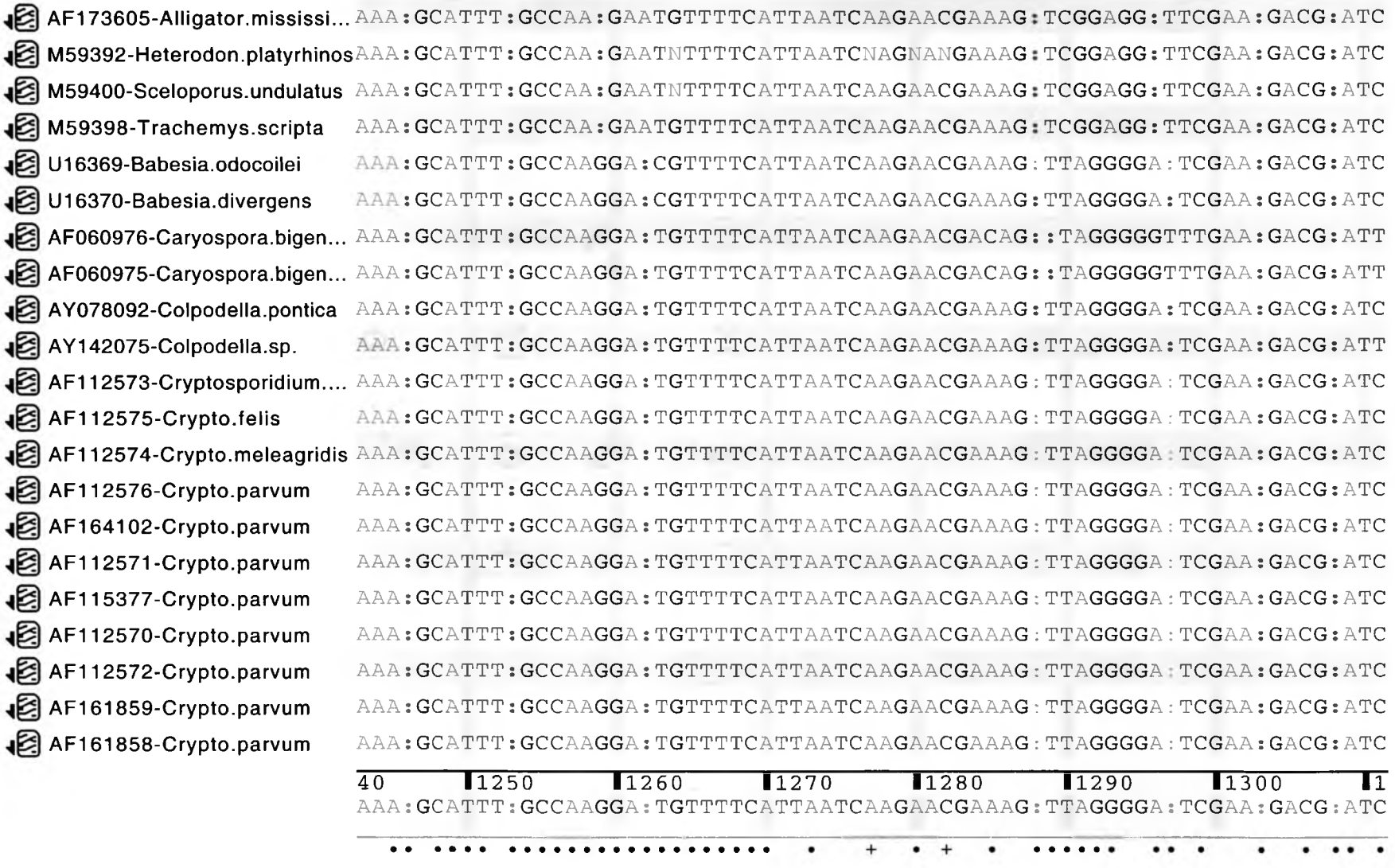

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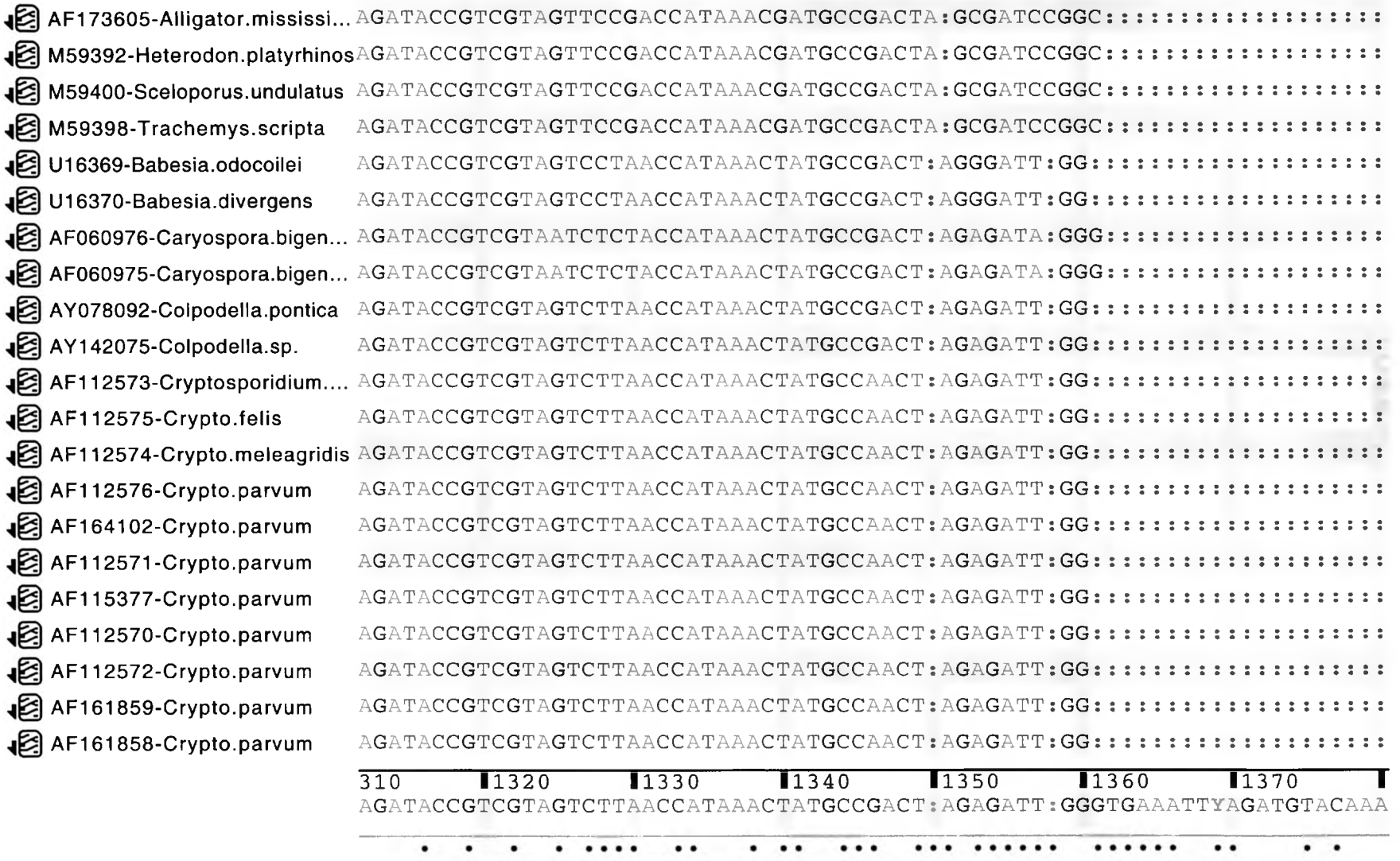

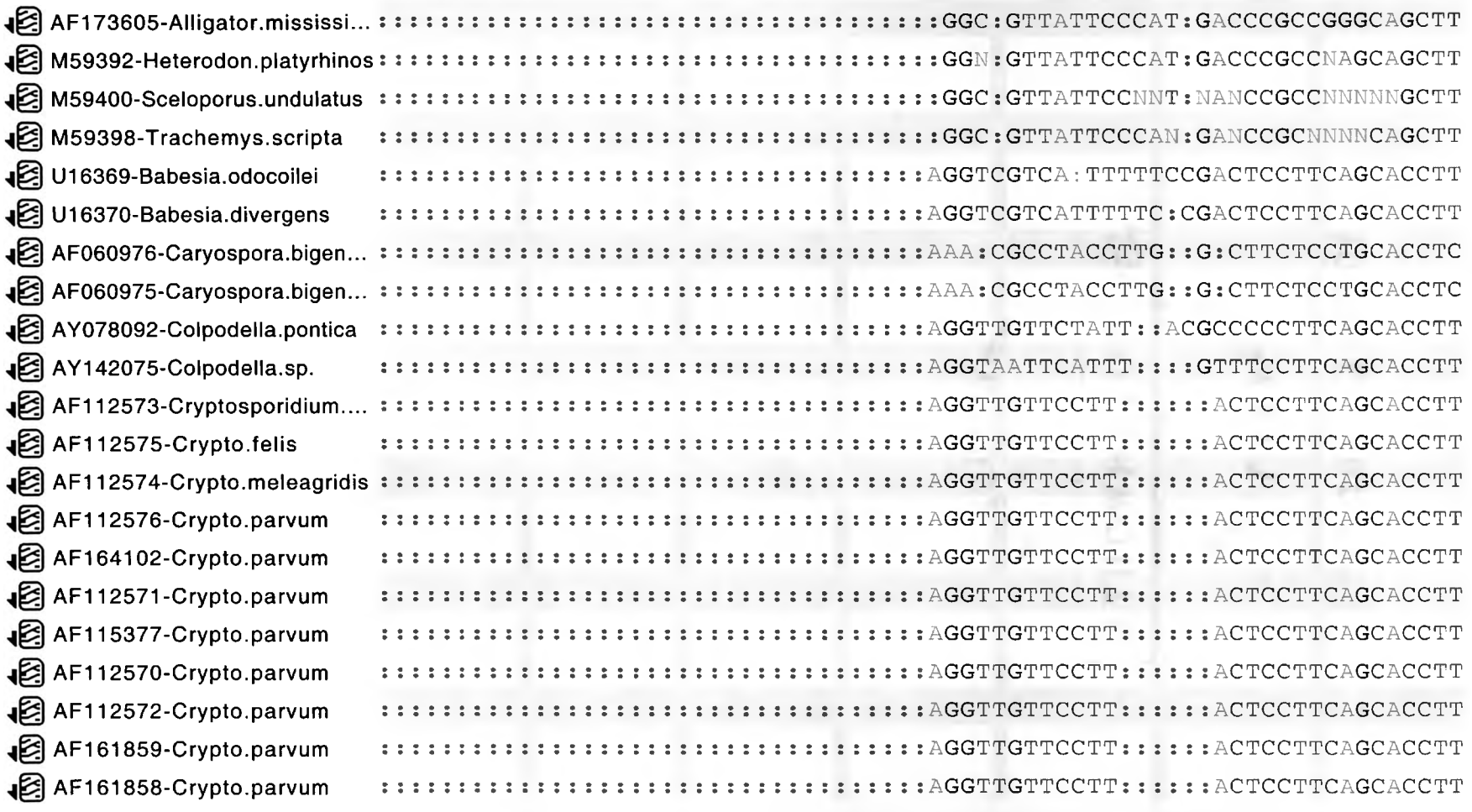
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U16370-Babesia.divergens
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AF060975-Caryospora.bigen...
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AY142075-Colpodella.sp.
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AF112576-Crypto.parvum
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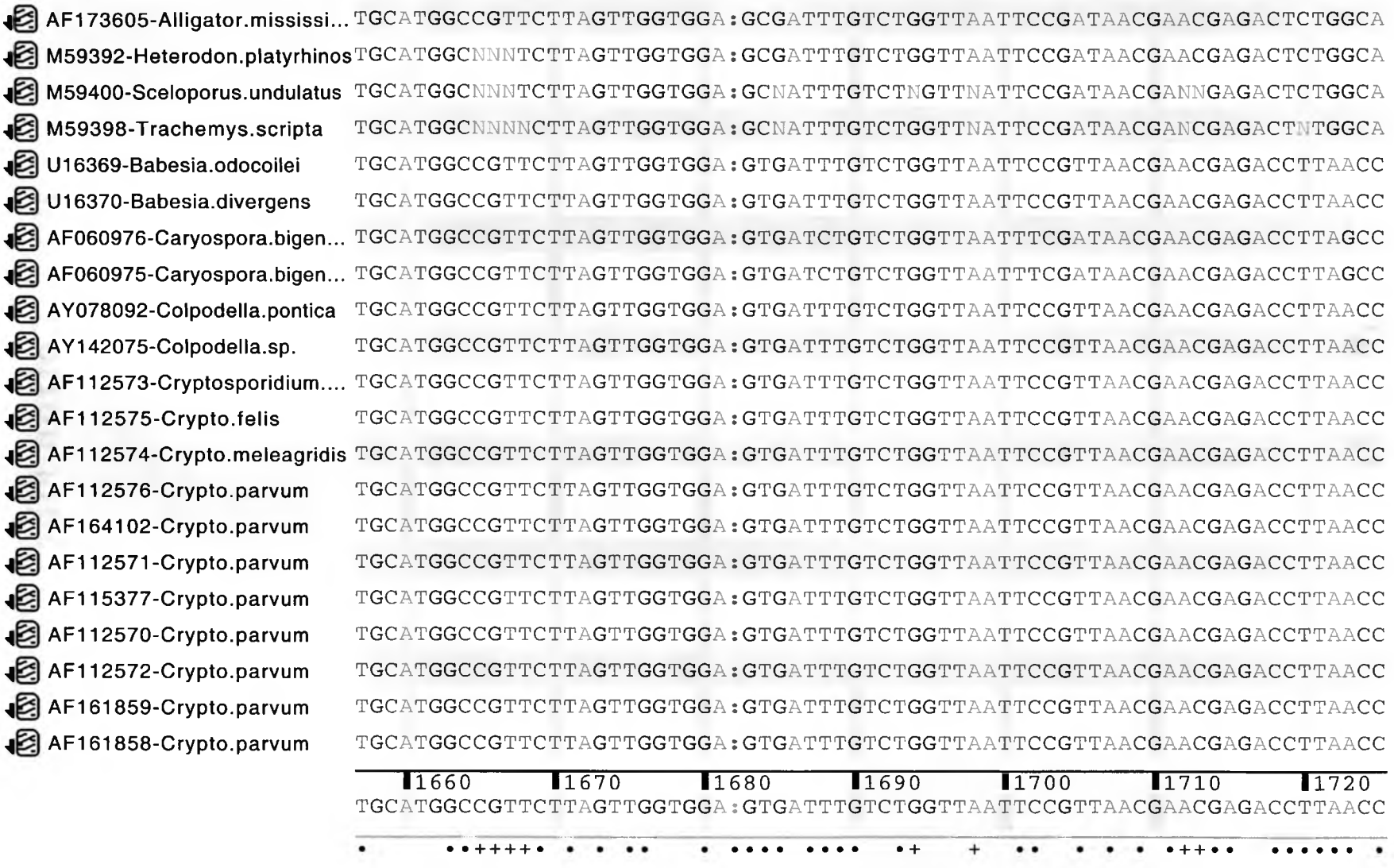
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Appendix C (cont.)

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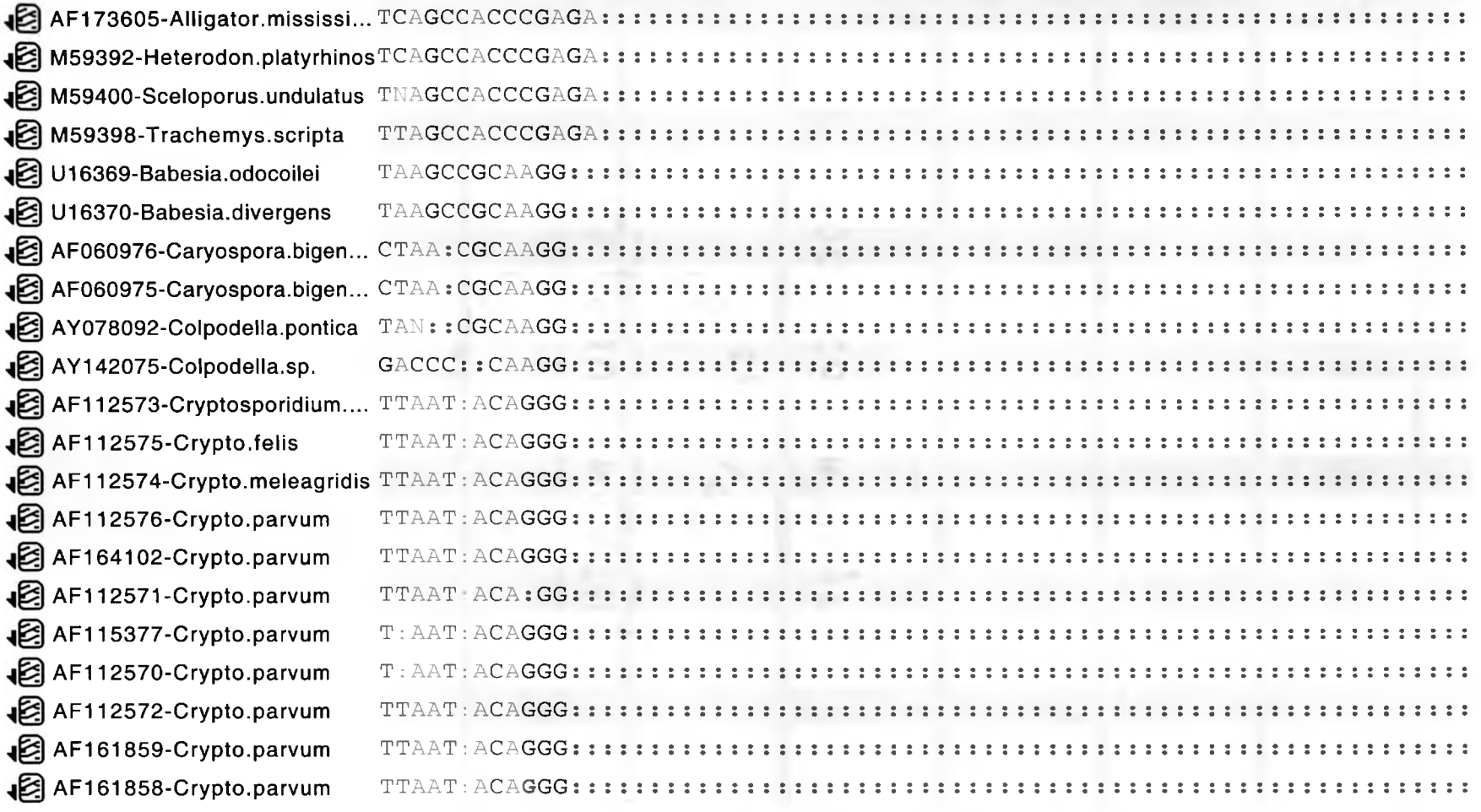
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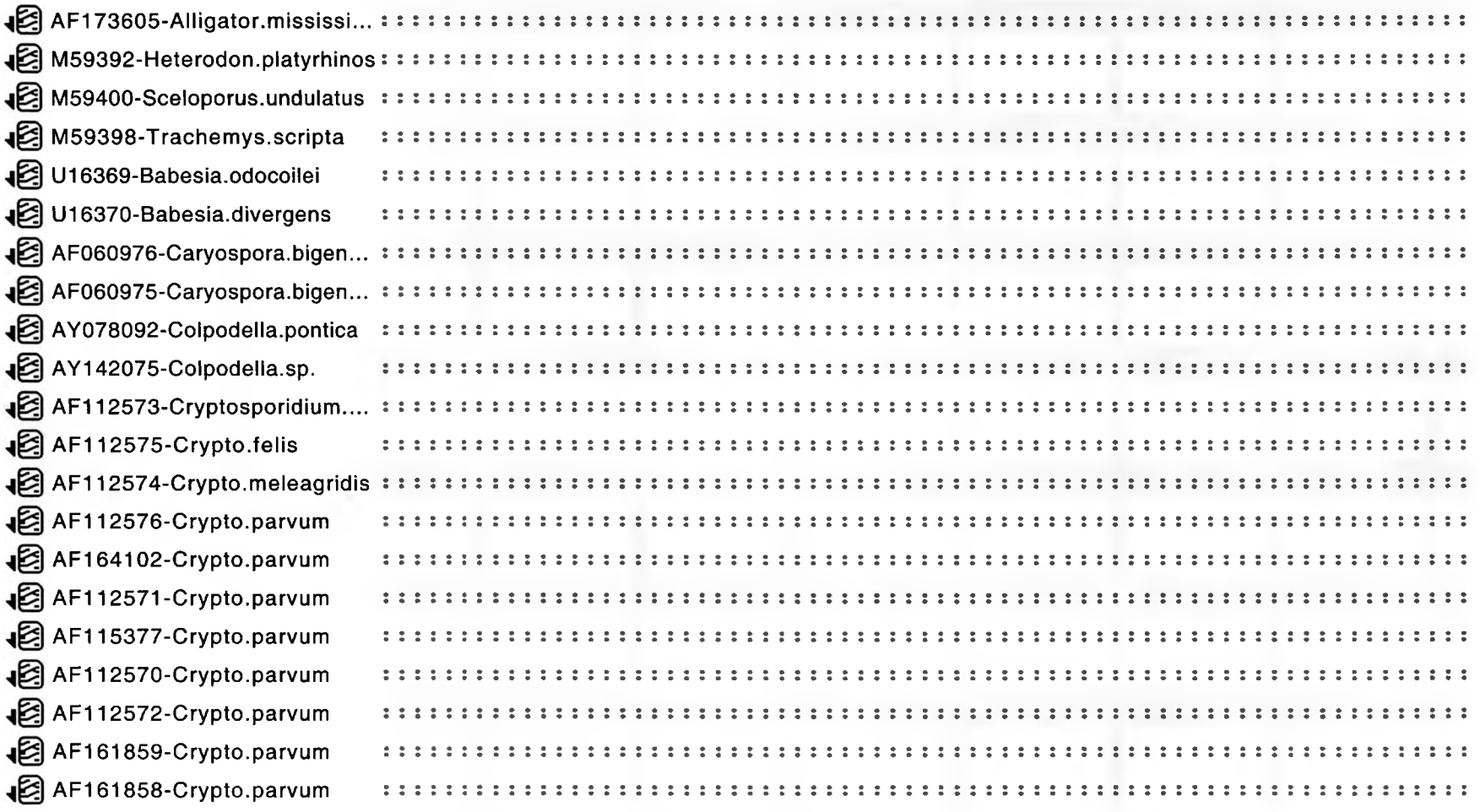
Appendix C (cont.)



Appendix C (cont.)

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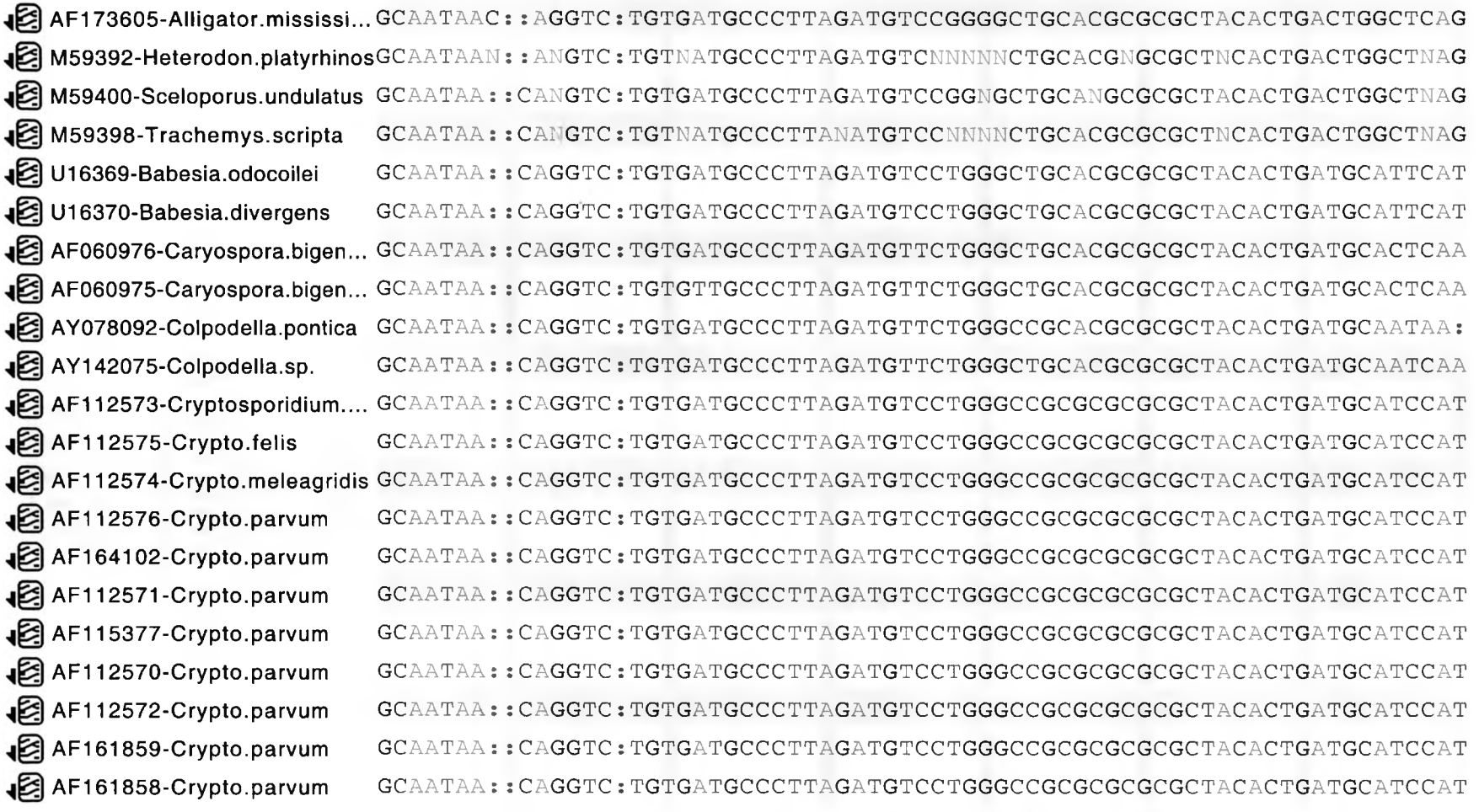

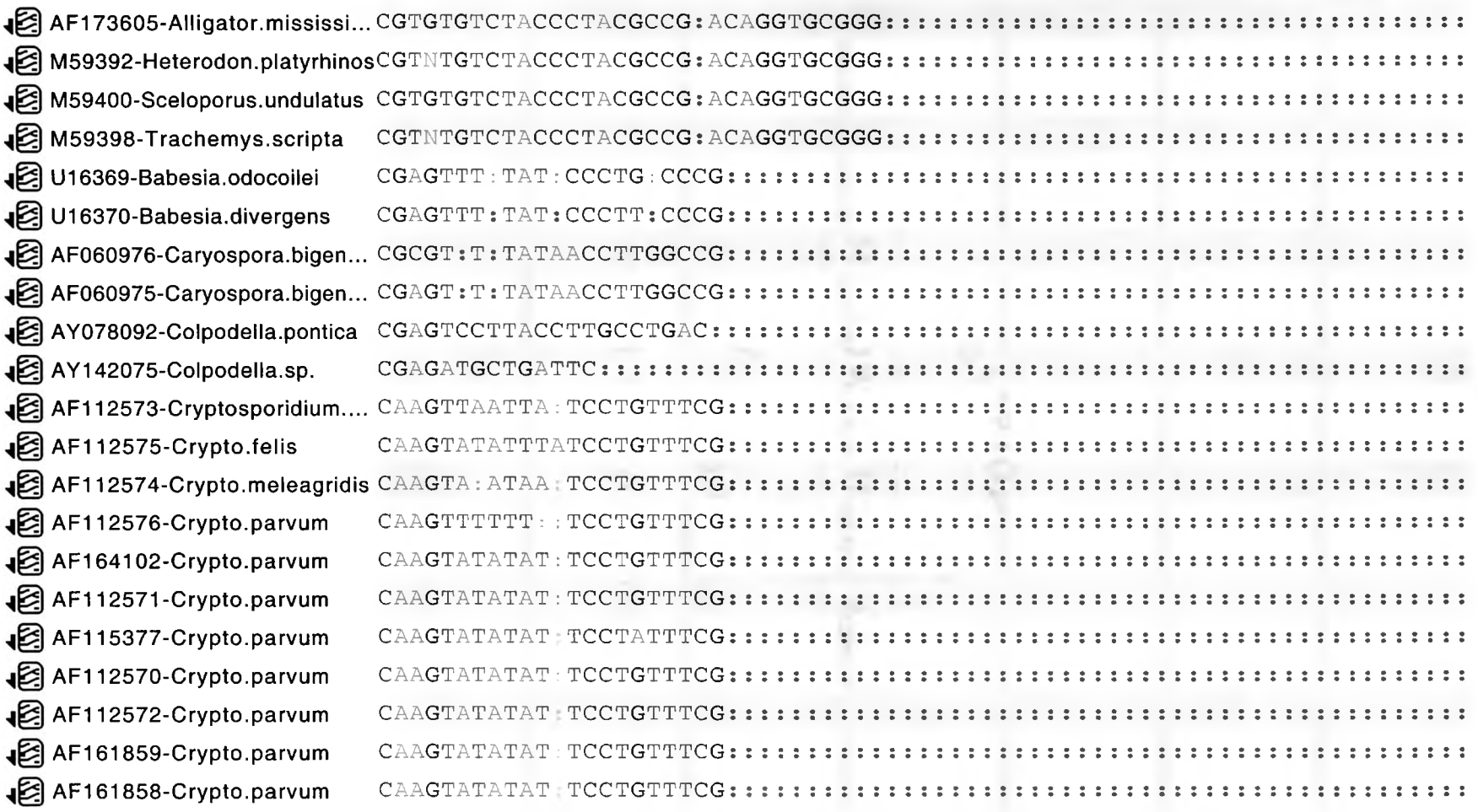
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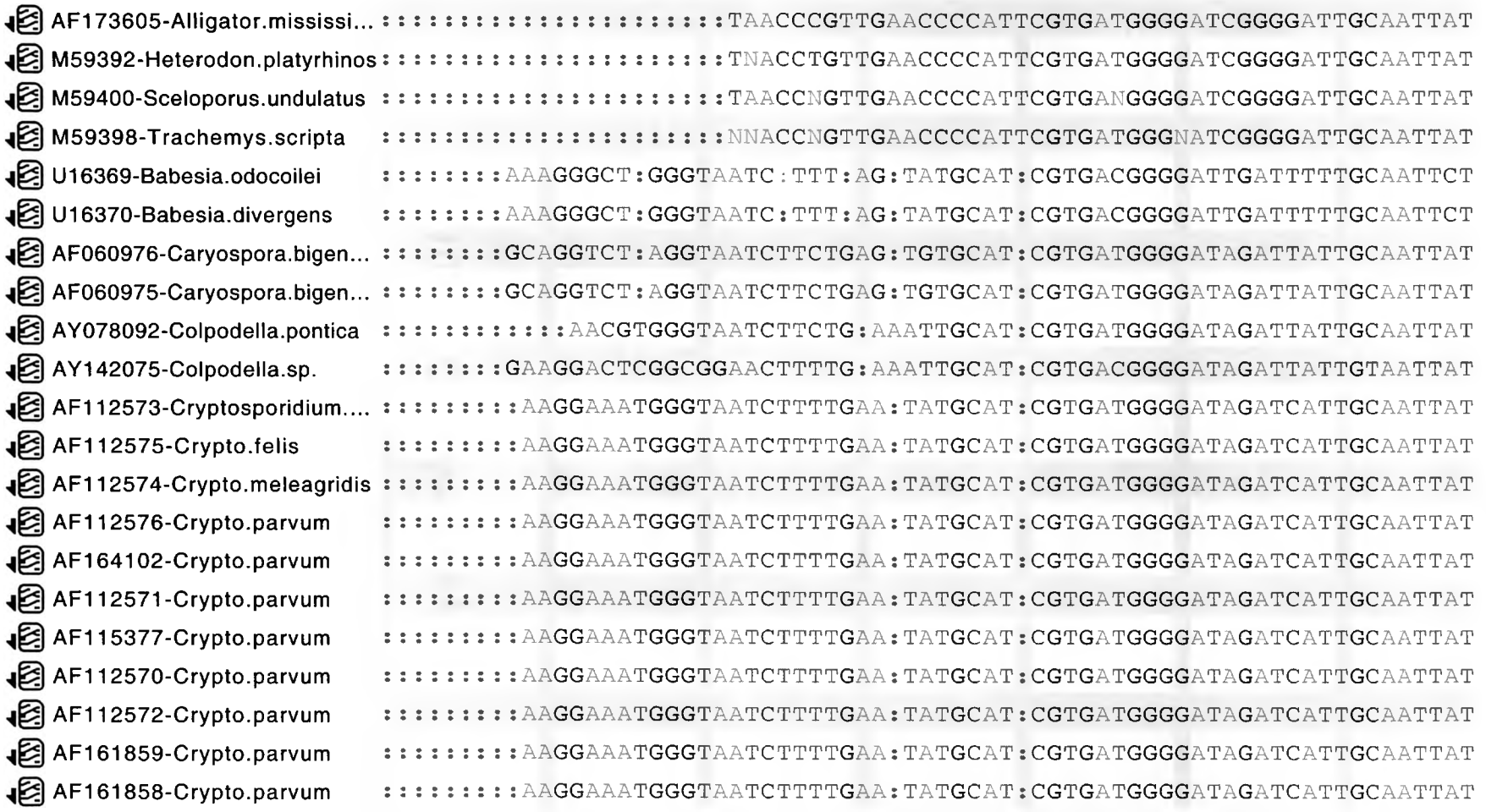
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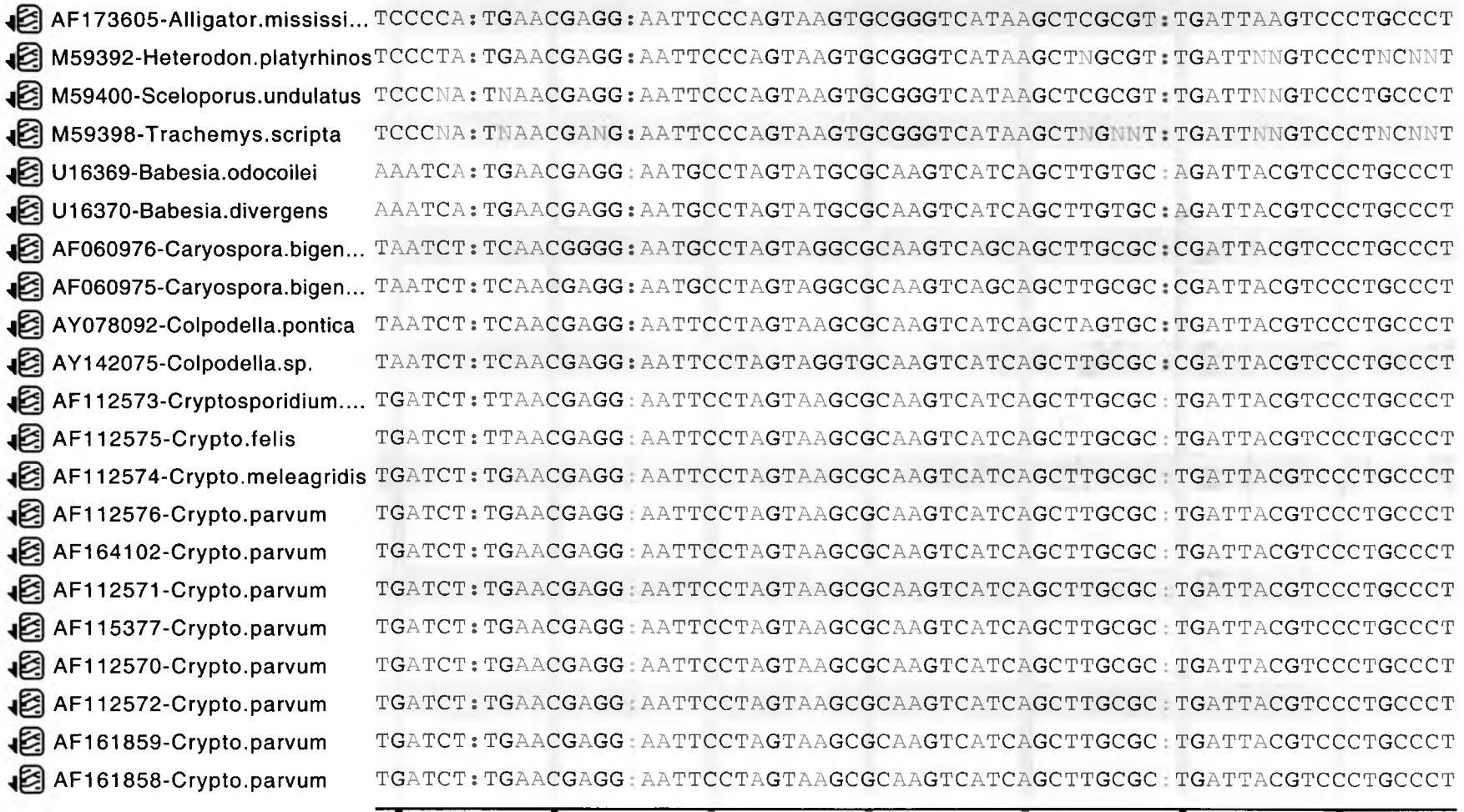
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Appendix C (cont.)

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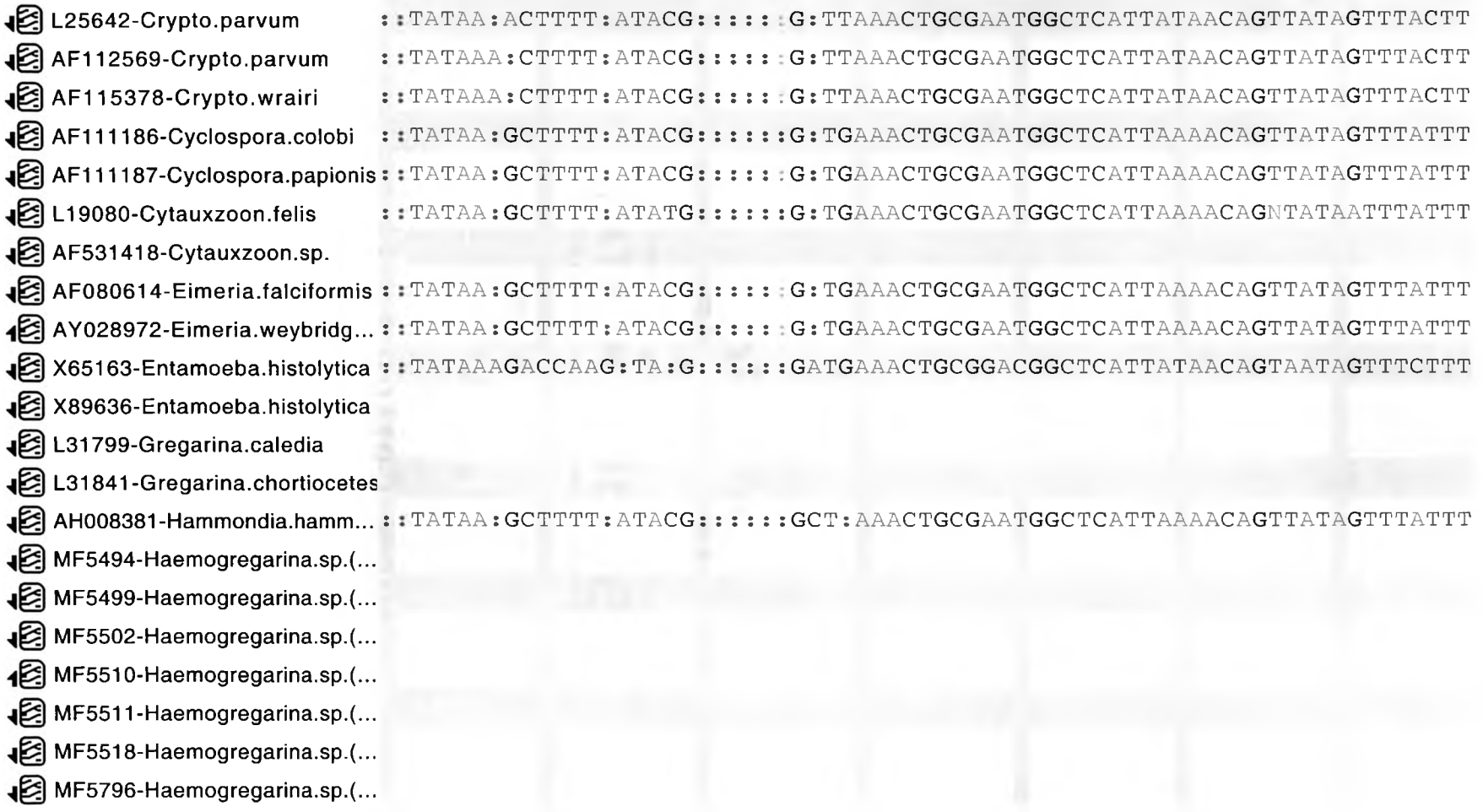
Appendix C (cont.)

Appendix C (cont.)



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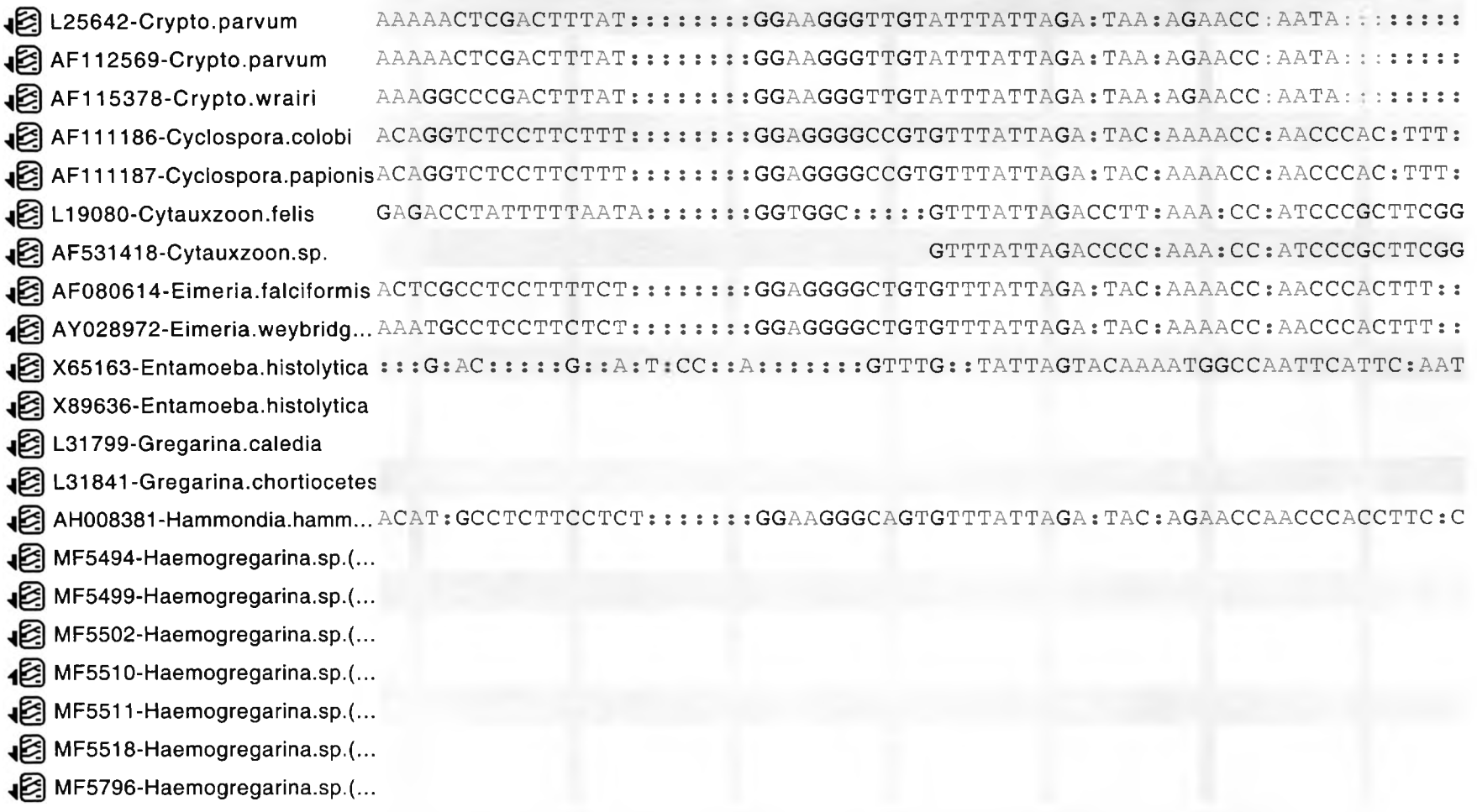
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X89636-Entamoeba.histolytica
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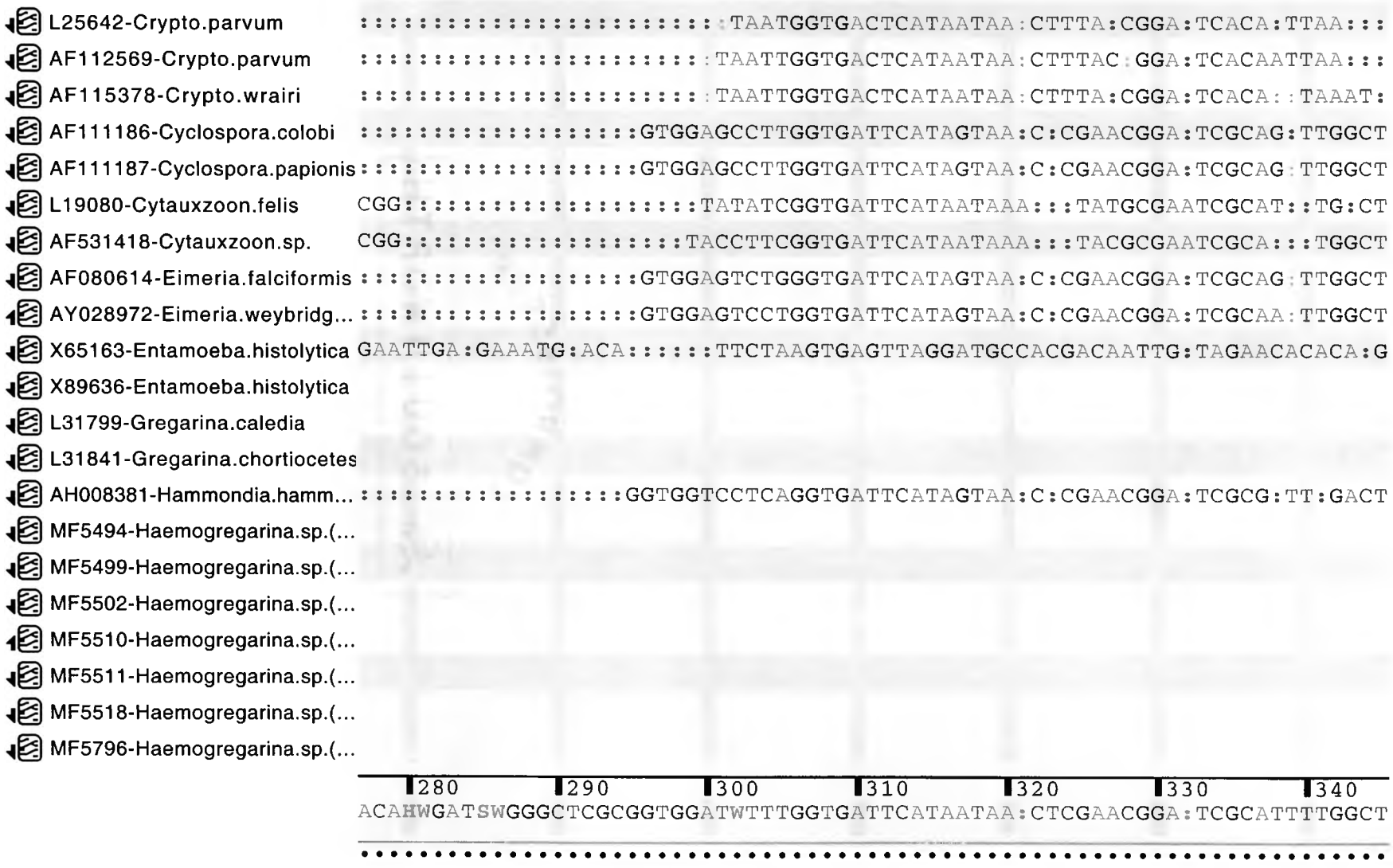


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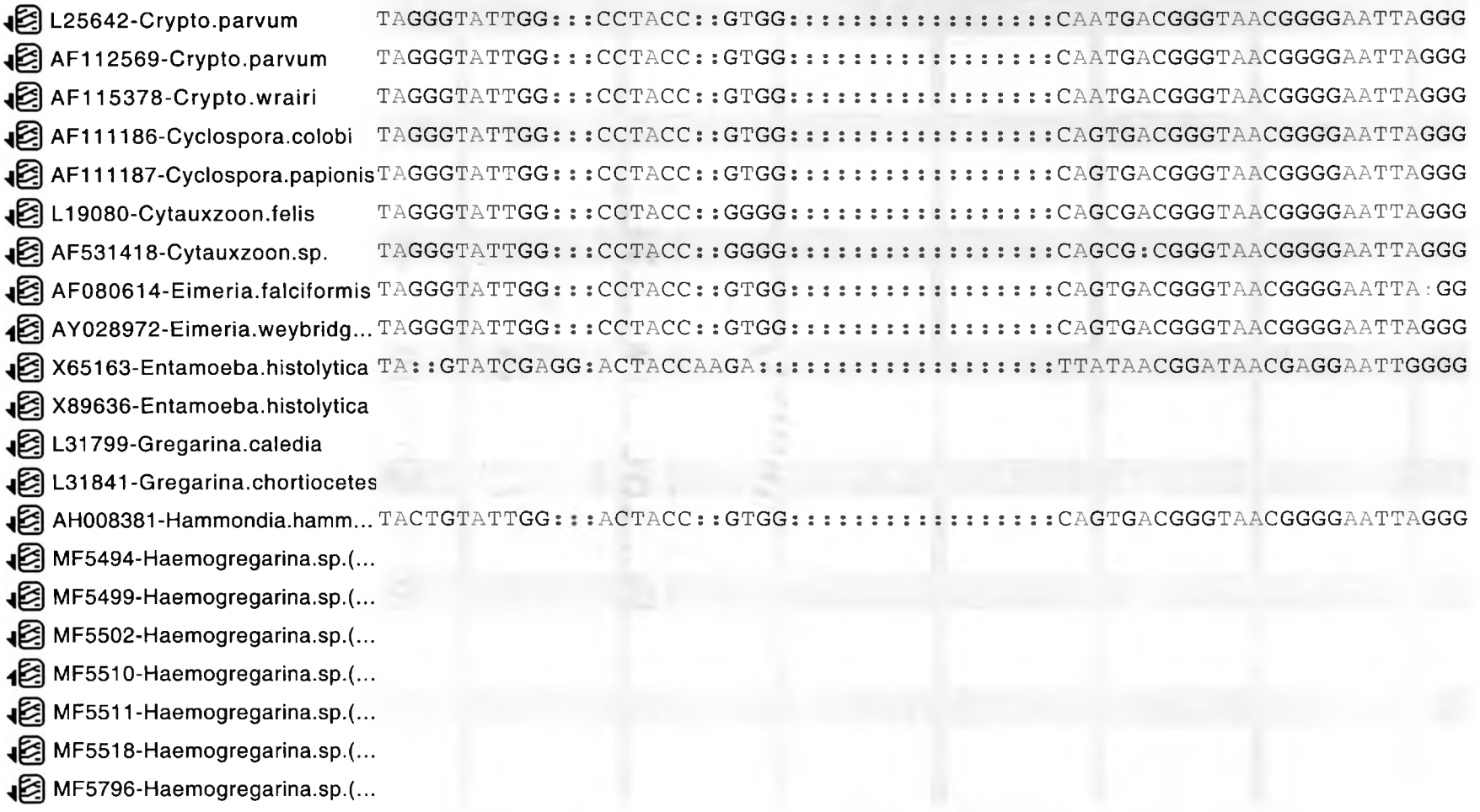
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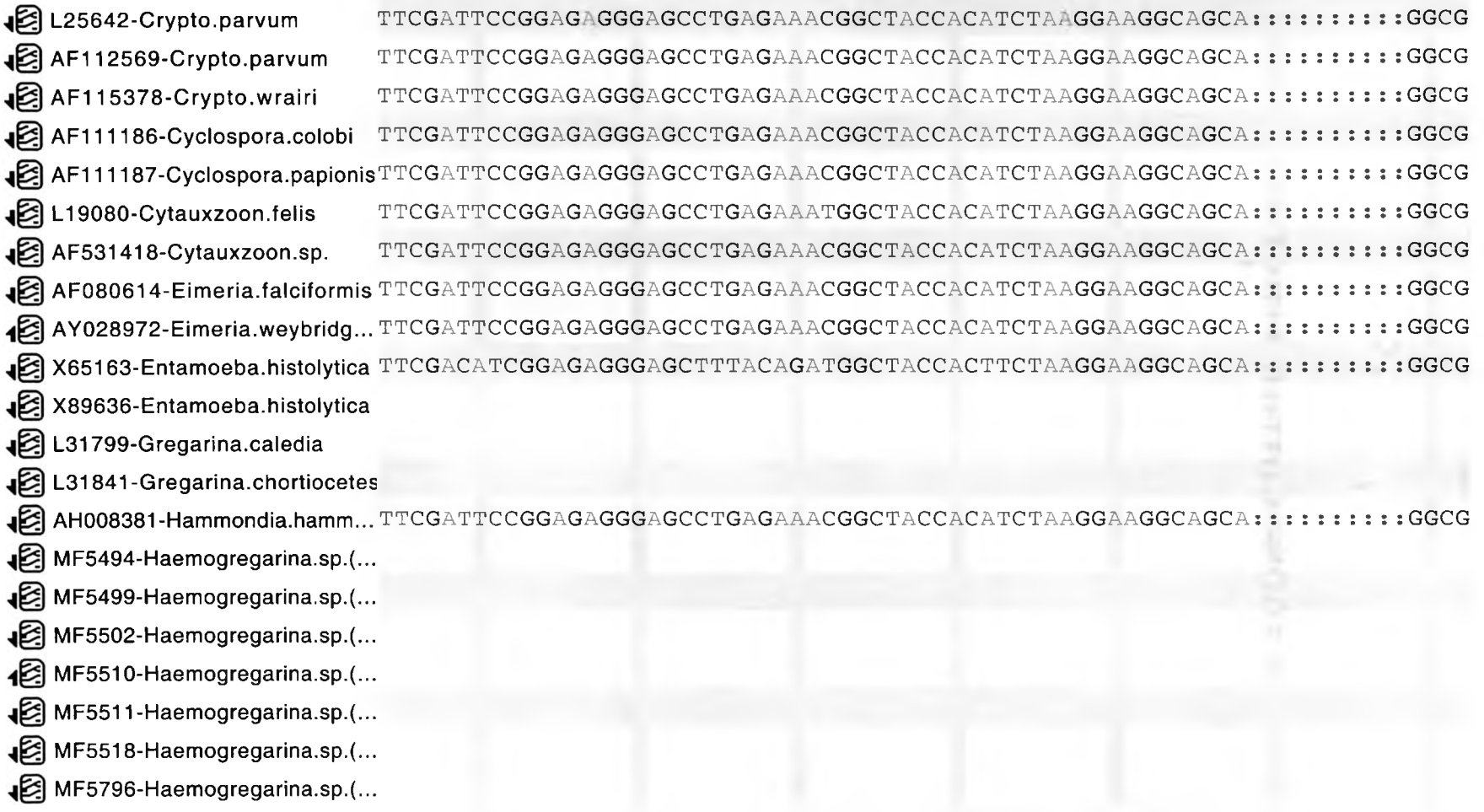
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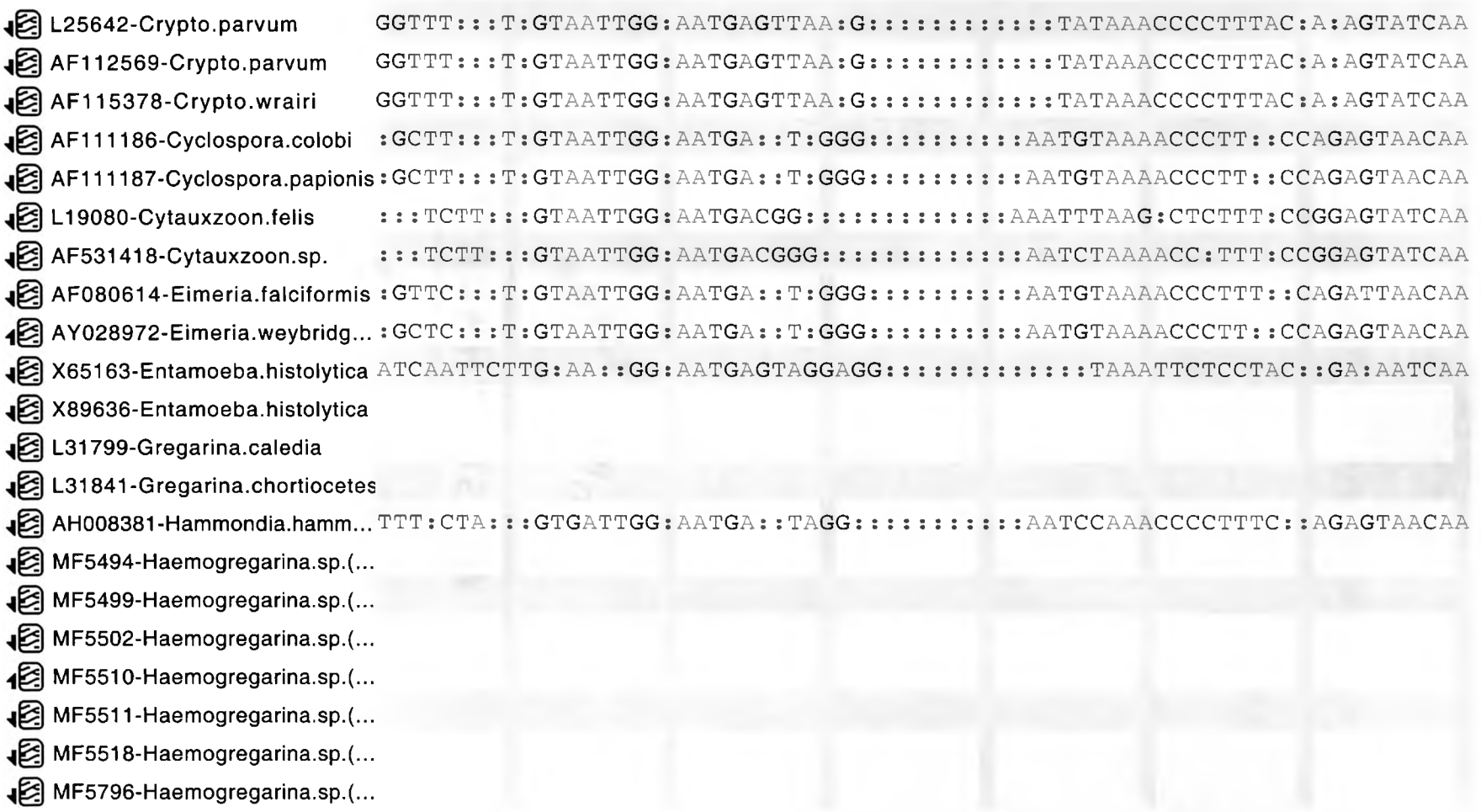
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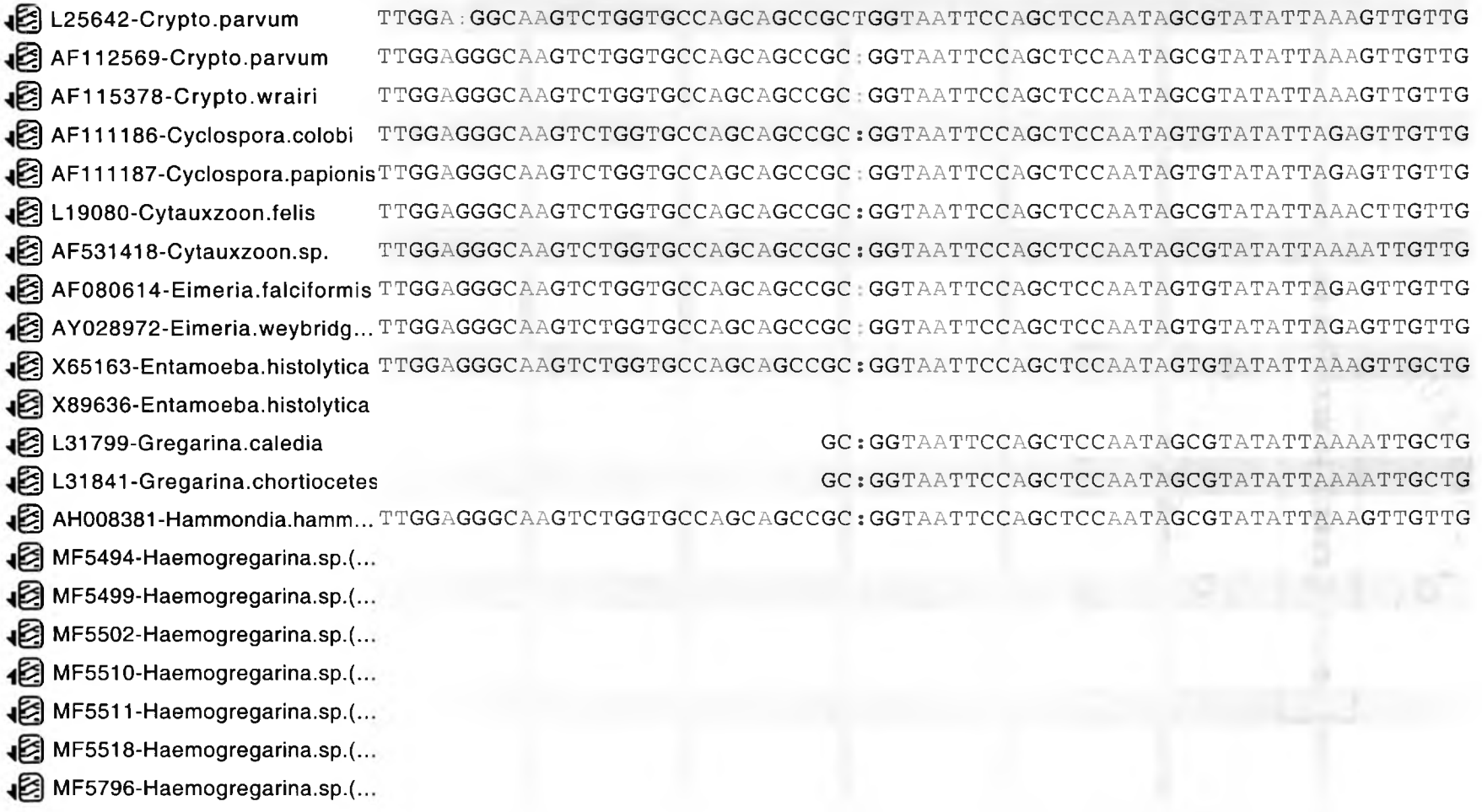
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X89636-Entamoeba.histolytica
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Appendix C (cont.)

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X89636-Entamoeba.histolytica	
L31799-Gregarina.caledia	CAGTTAAAGCGTCCGTAGTTGAATTTC:::
L31841-Gregarina.chortiocetes	CAGTTAAAGCGTCCGTAGTTGGATTTC:::
AH008381-Hammondia.hamm...	CAGTTAAAAAGCTCGTAGTTGGATTTCT::
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MF5499-Haemogregarina.sp.(...	
MF5502-Haemogregarina.sp.(...	
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MF5796-Haemogregarina.sp.(...	

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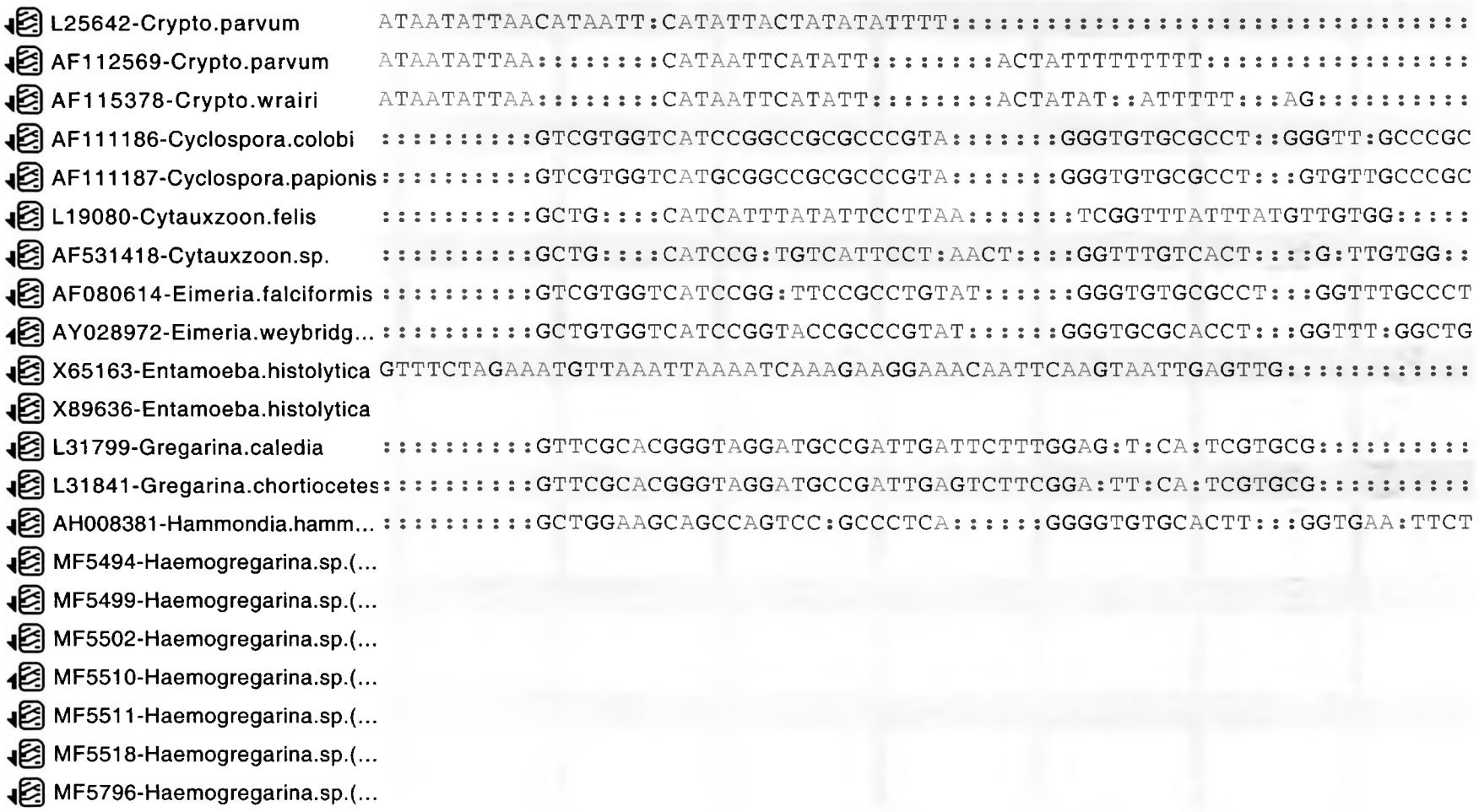
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AF531418-Cytauxzoon.sp.
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X89636-Entamoeba.histolytica
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MF5511-Haemogregarina.sp.(...
MF5518-Haemogregarina.sp.(...
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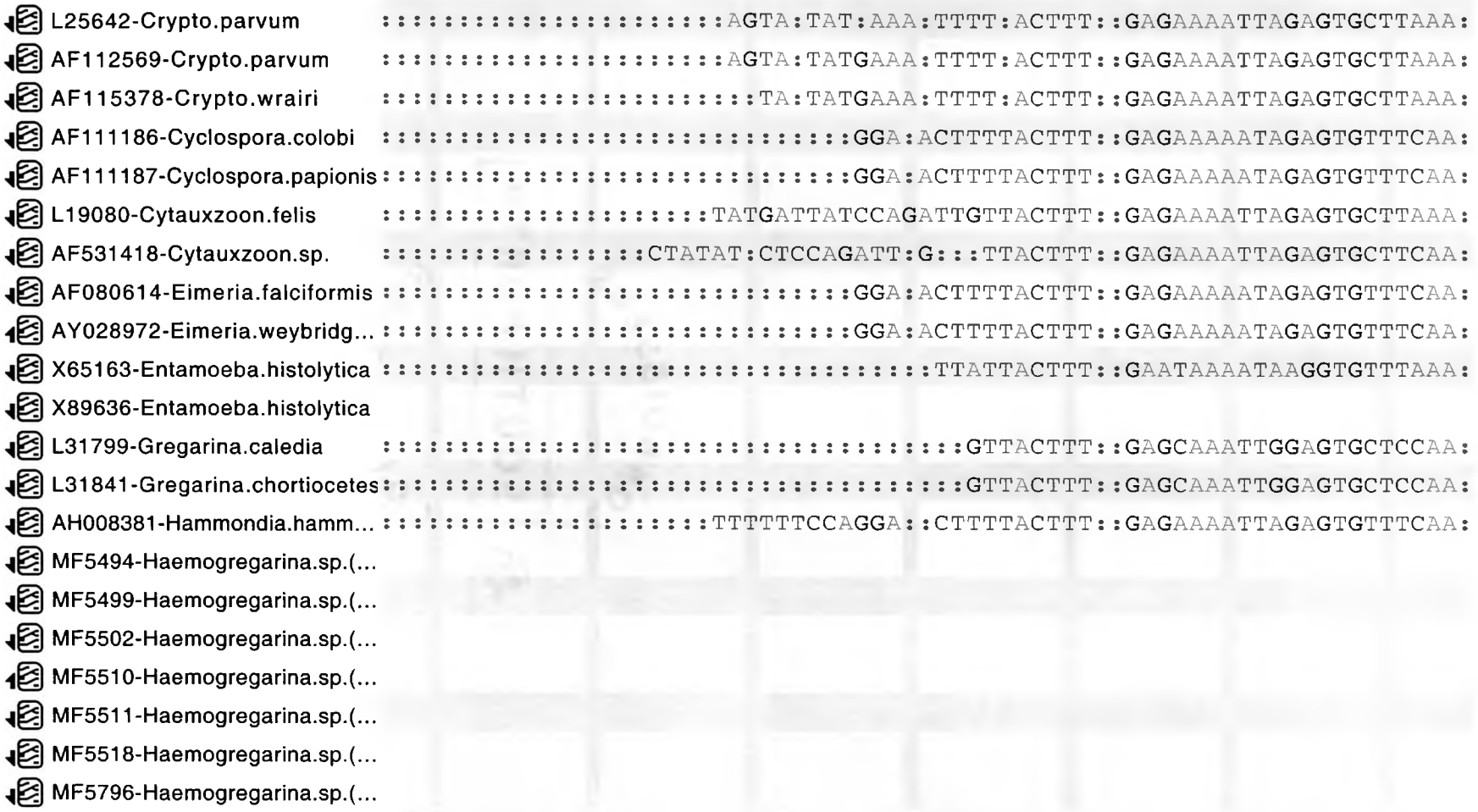
Appendix C (cont.)

Appendix C (cont.)

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X89636-Entamoeba.histolytica
L31799-Gregarina.caledia CCAGGCTT:A:CG::::::::::::::::C:TT:GAACAGCTCAGCATGGAA:TAACAA:GATAGGACT
L31841-Gregarina.chortiocetes CCAGGCTT:A:CG::::::::::::::::C:TT:GAACAGCTCAGCATGGAA:TAACAA:GATAGGACT
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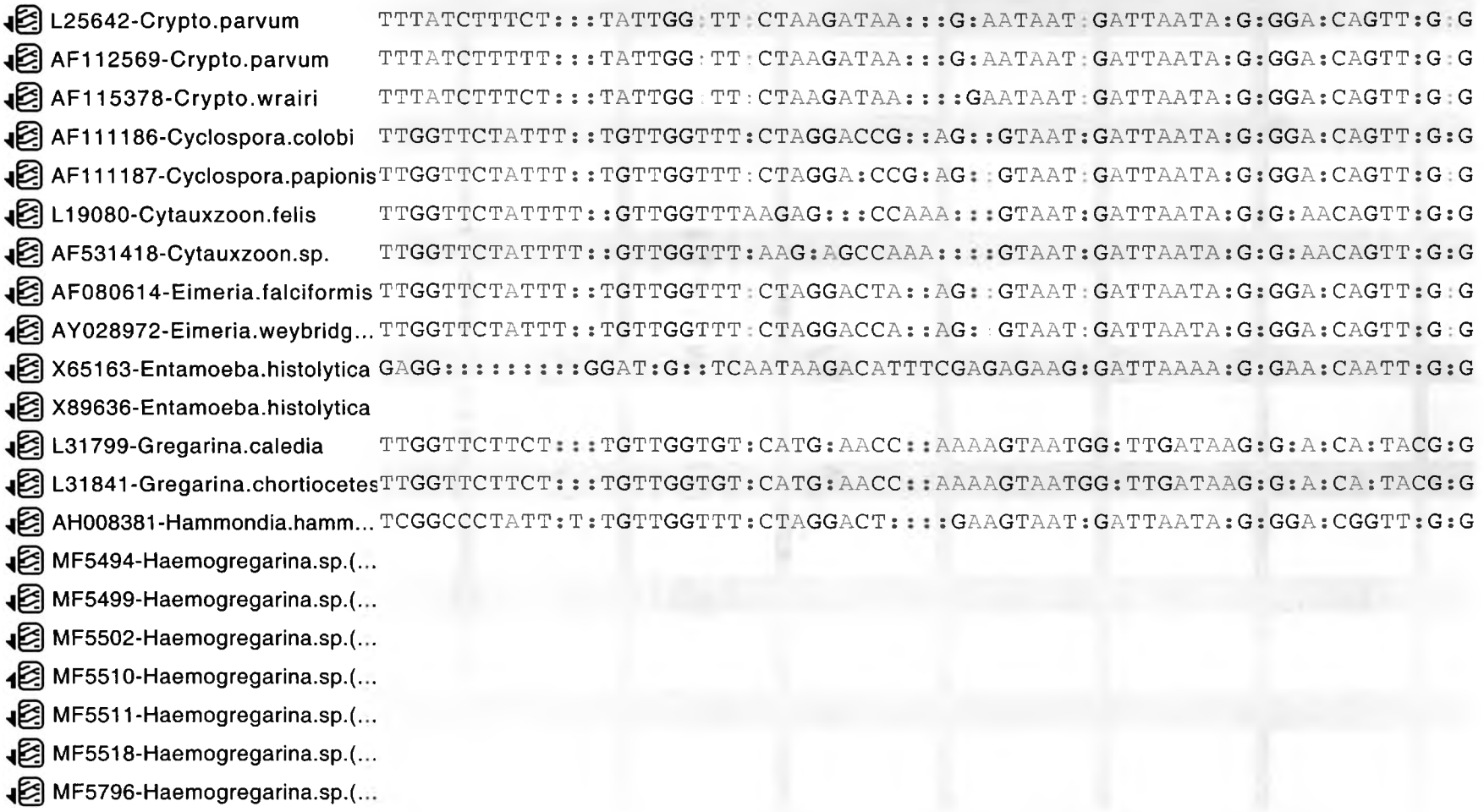
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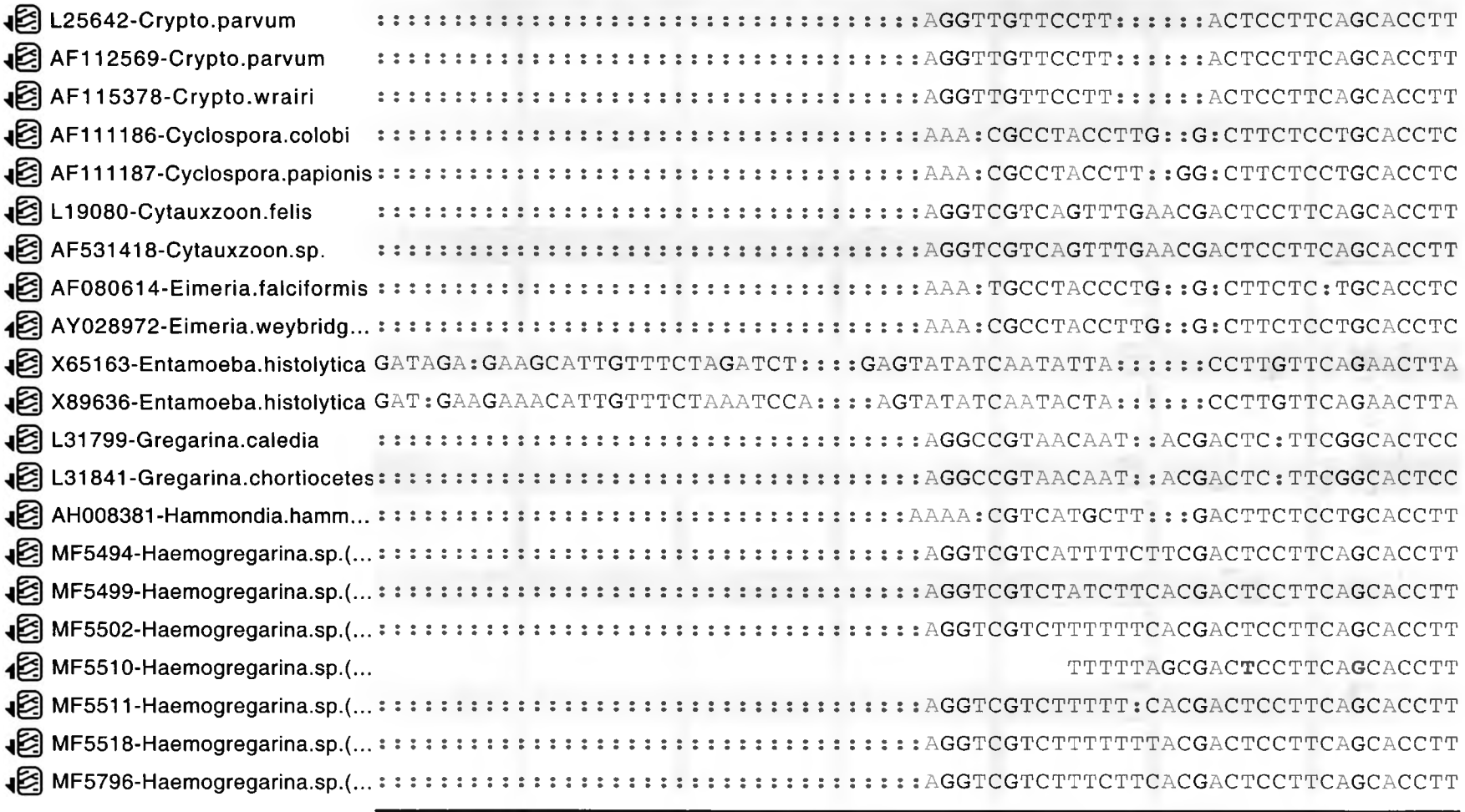
Appendix C (cont.)

Appendix C (cont.)

Appendix C (cont.)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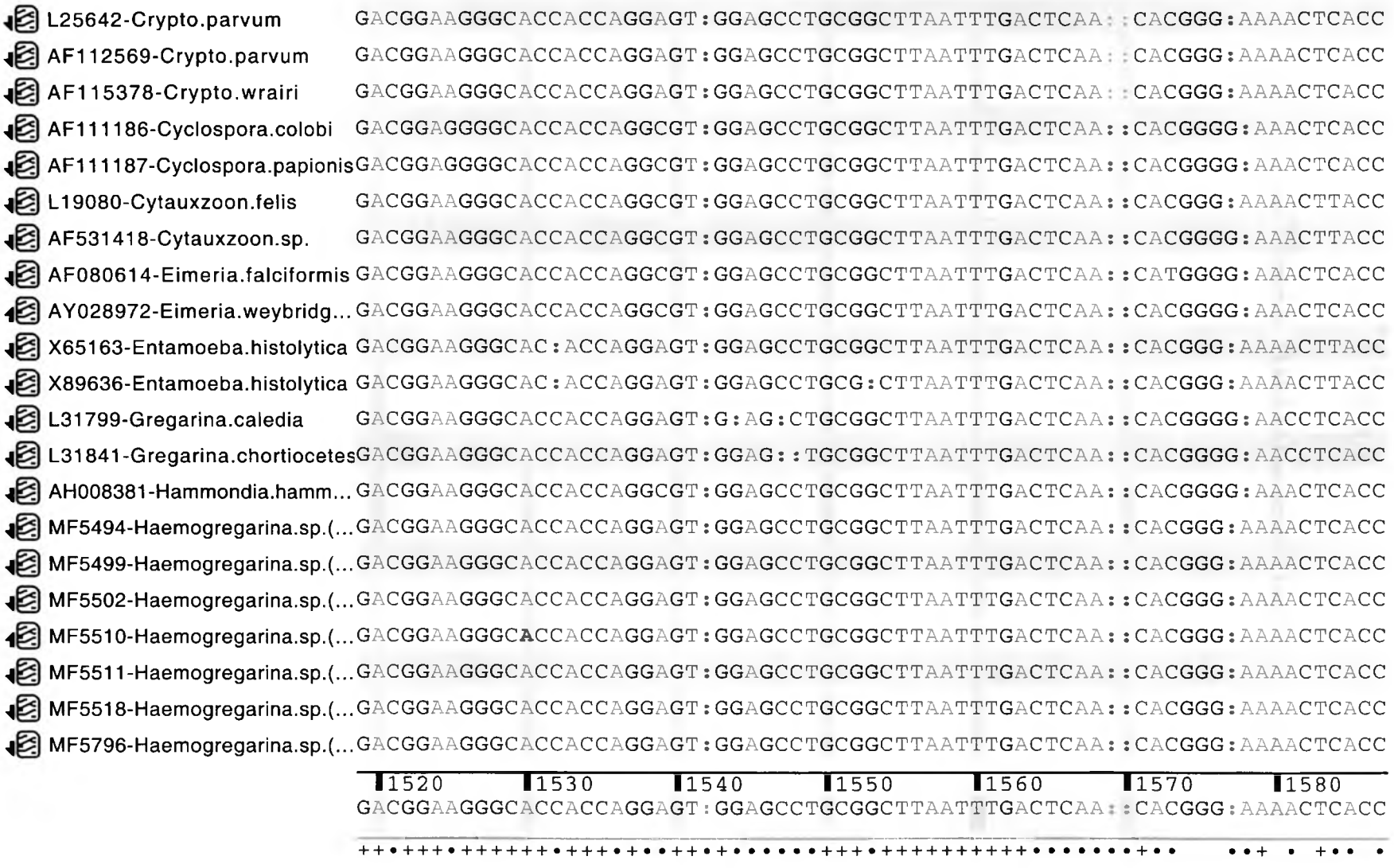
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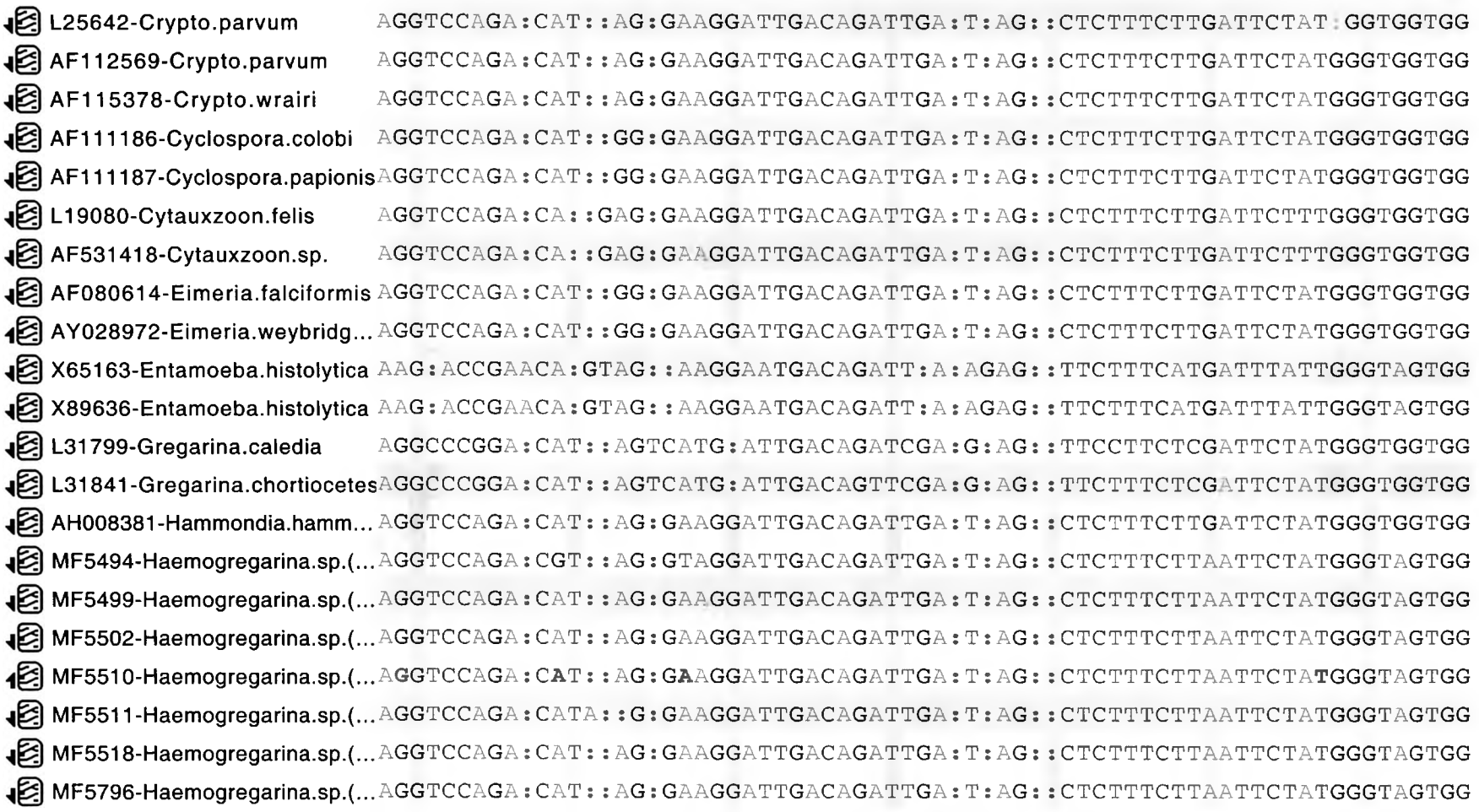
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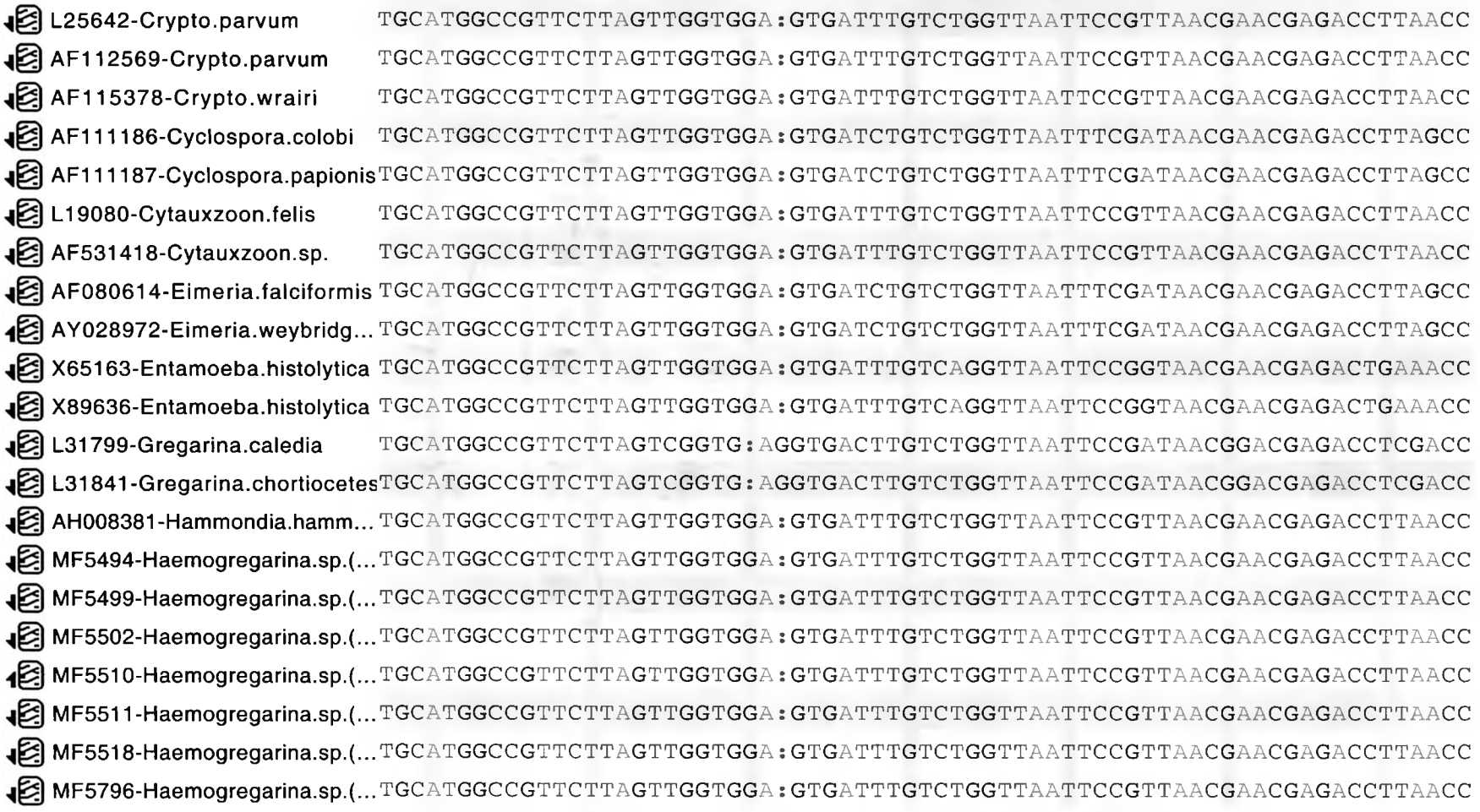
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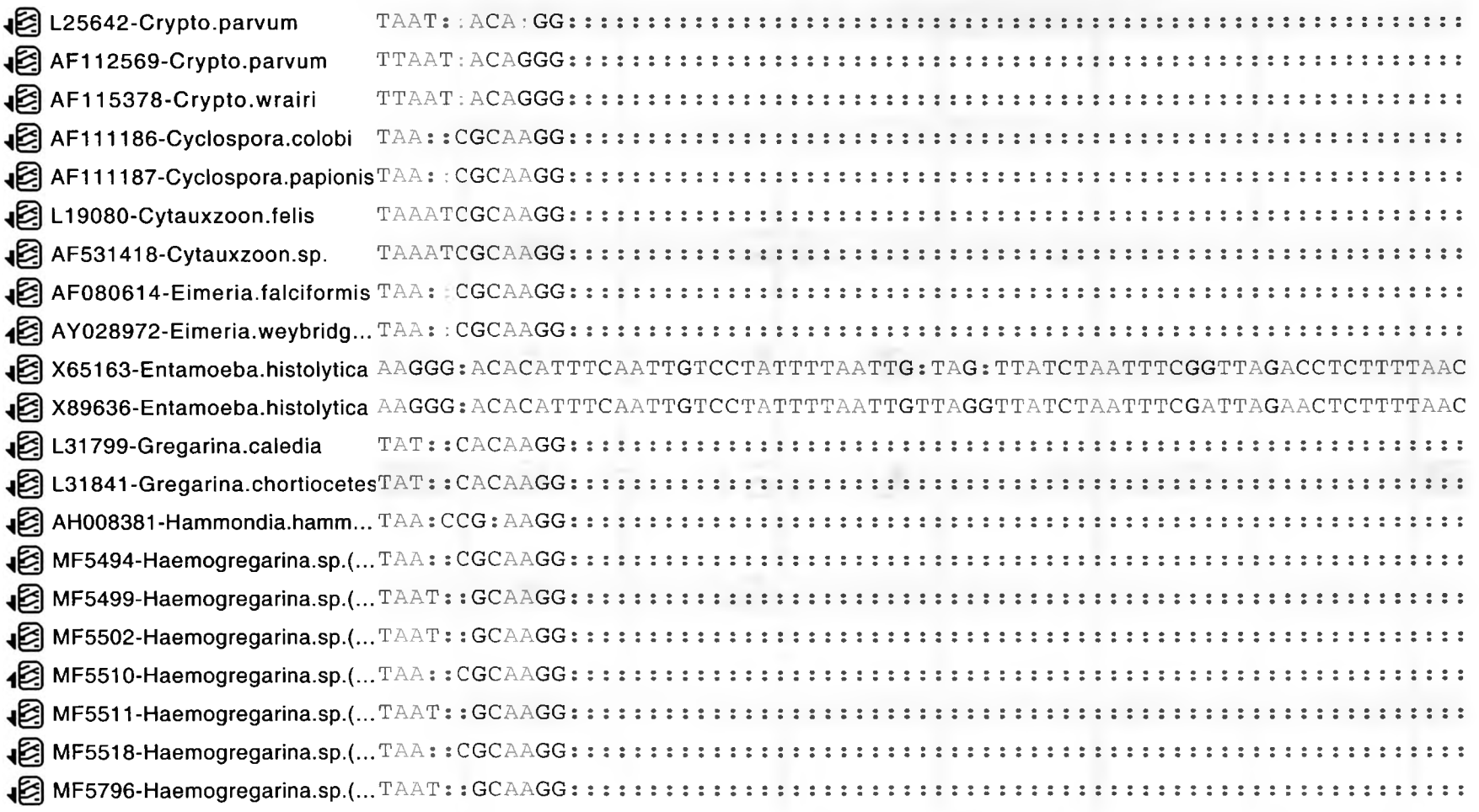
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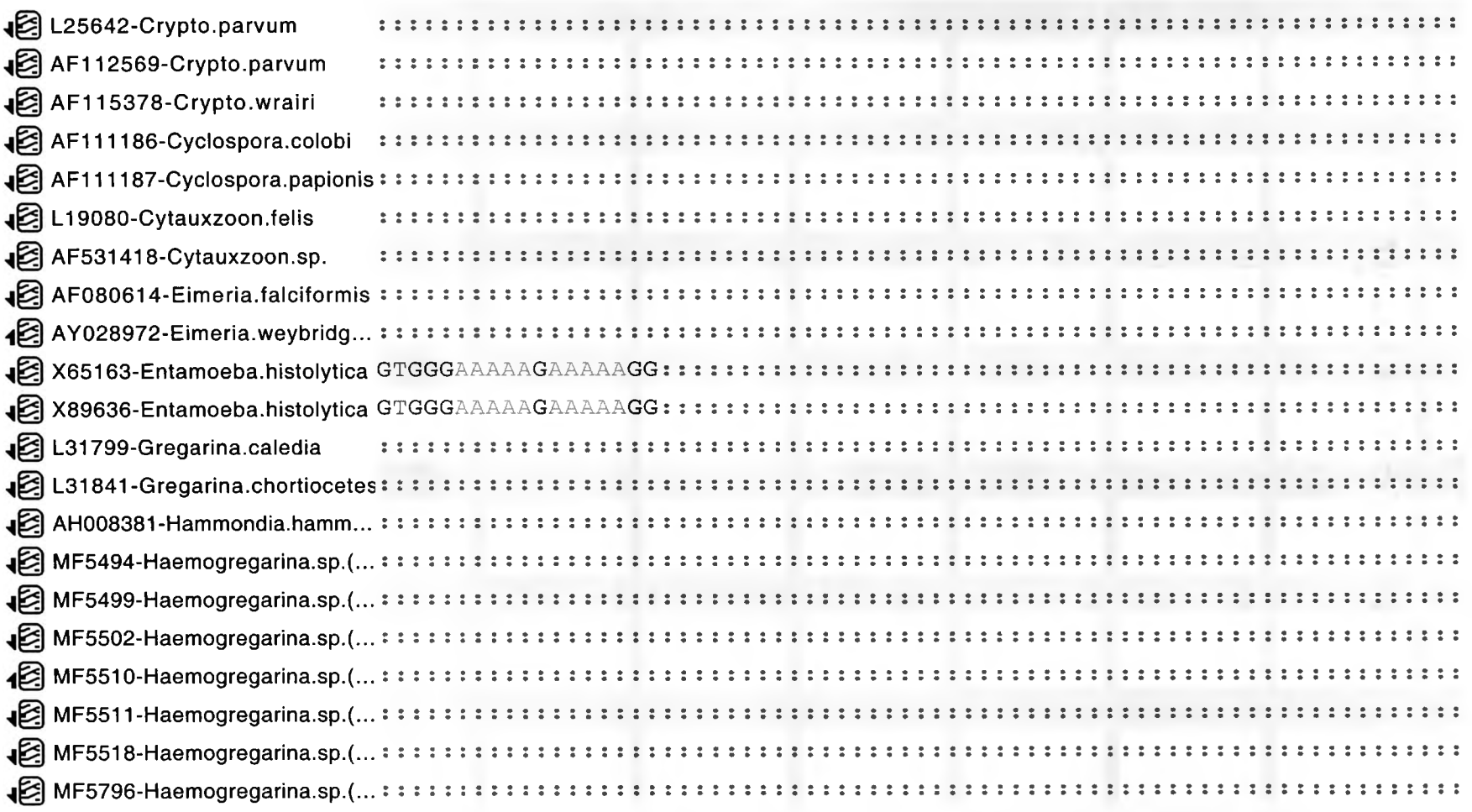
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L19080-Cytauxzoon.felis
AF531418-Cytauxzoon.sp.
AF080614-Eimeria.falciformis
AY028972-Eimeria.weybridg...
X65163-Entamoeba.histolytica GTGGGAAAAAGAAAAAGG
X89636-Entamoeba.histolytica GTGGGAAAAAGAAAAAGG
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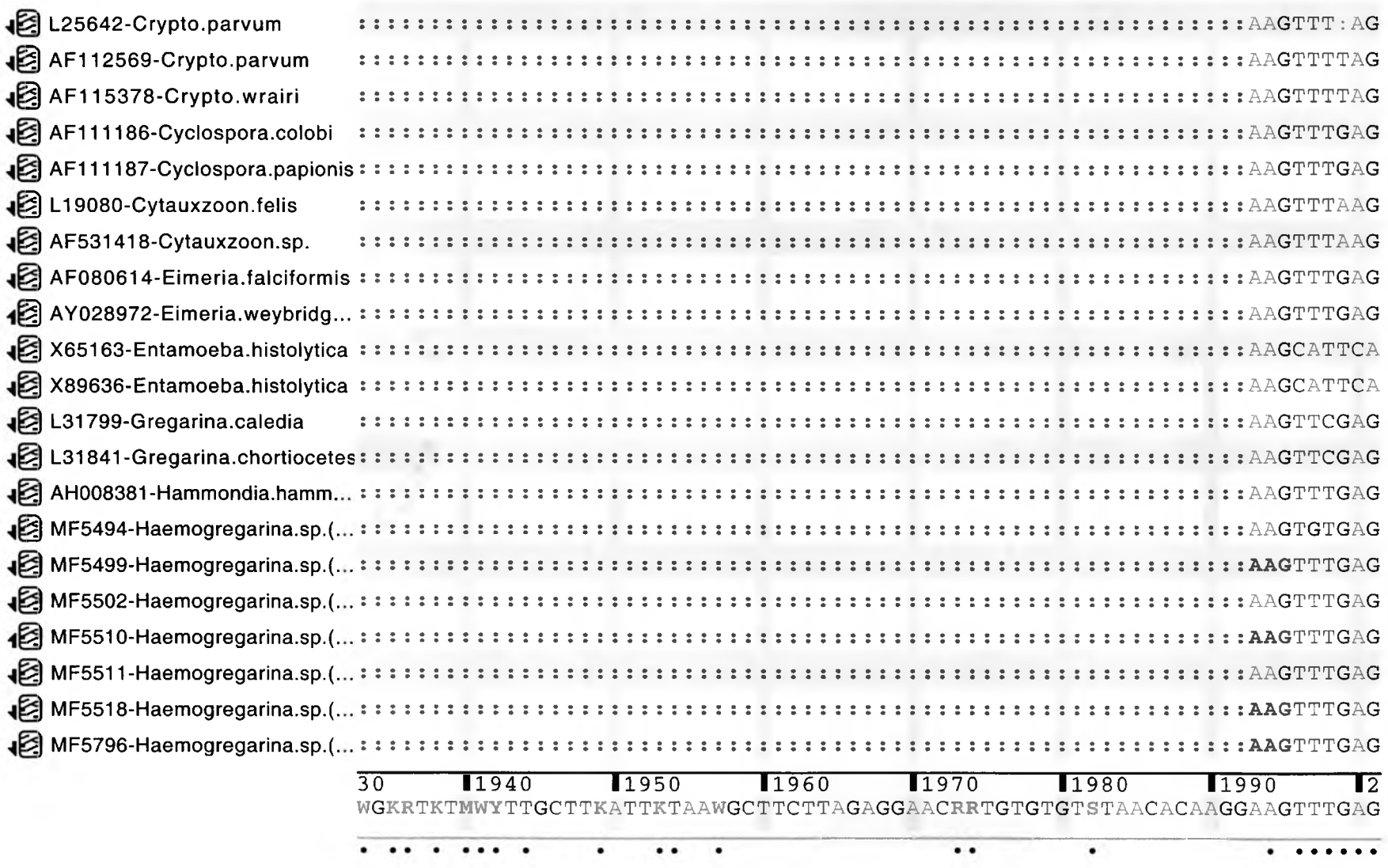
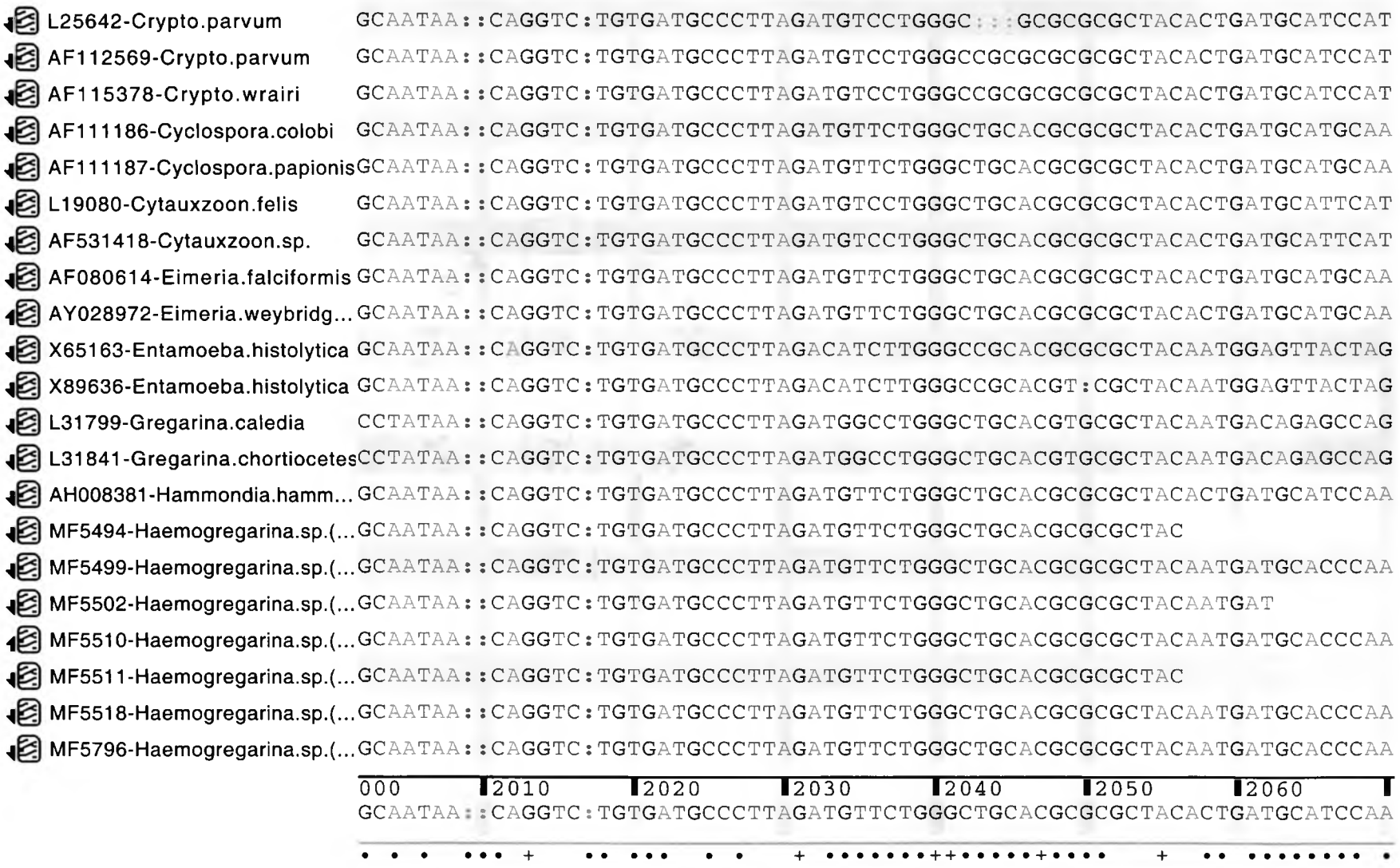
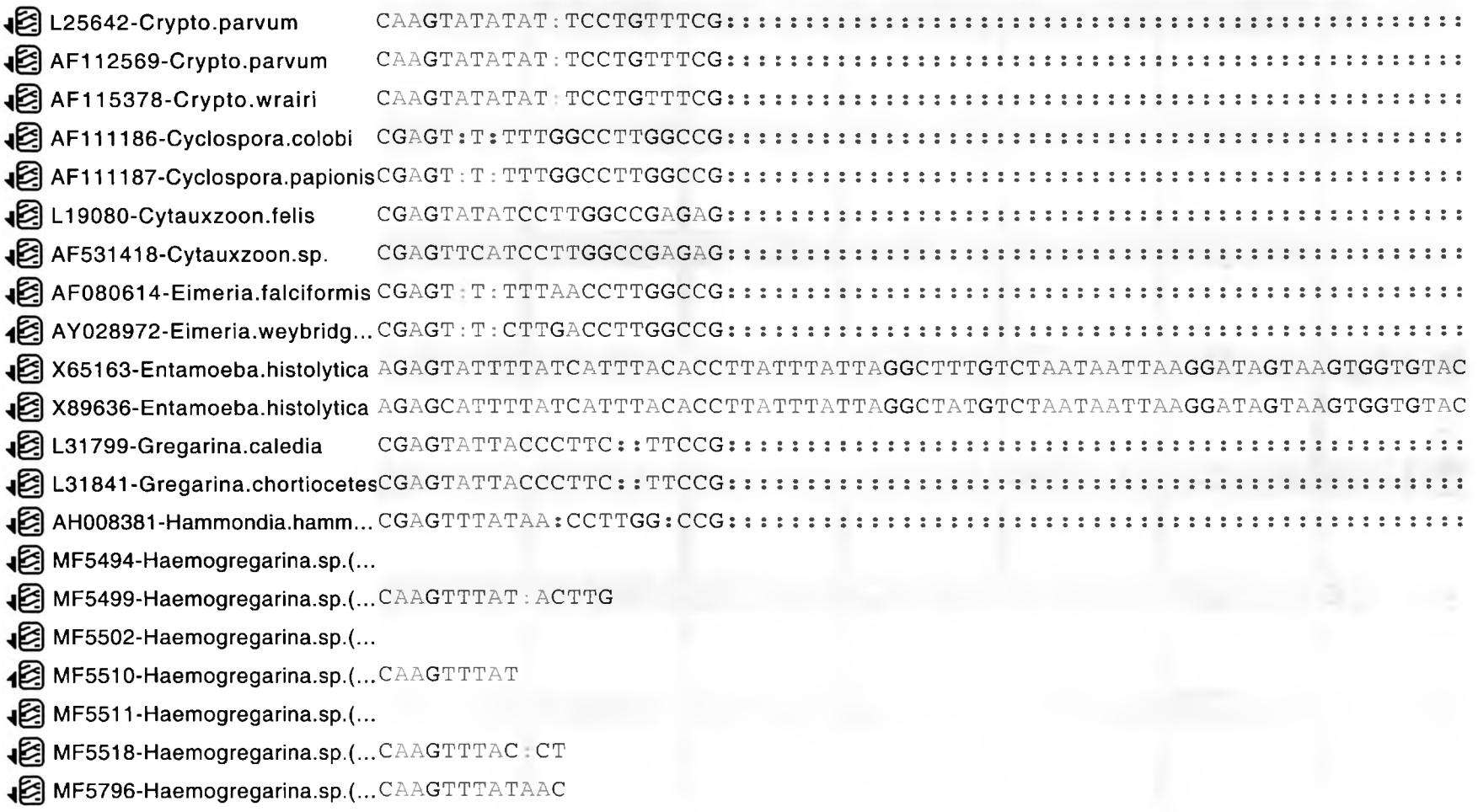
Appendix C (cont.)



Appendix C (cont.)

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AF080614-Eimeria.falciformis CGAGT:T:TTTAACCTTGGCCG:::
AY028972-Eimeria.weybridg... CGAGT:T:CTTGACCTTGGCCG:::
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X89636-Entamoeba.histolytica AGAGCATTTTATCATTTACACCTTATTTATTAGGCTATGTCTAATAATTAAGGATAGTAAGTGGTGTAC
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MF5510-Haemogregarina.sp.(... CAAGTTTAT
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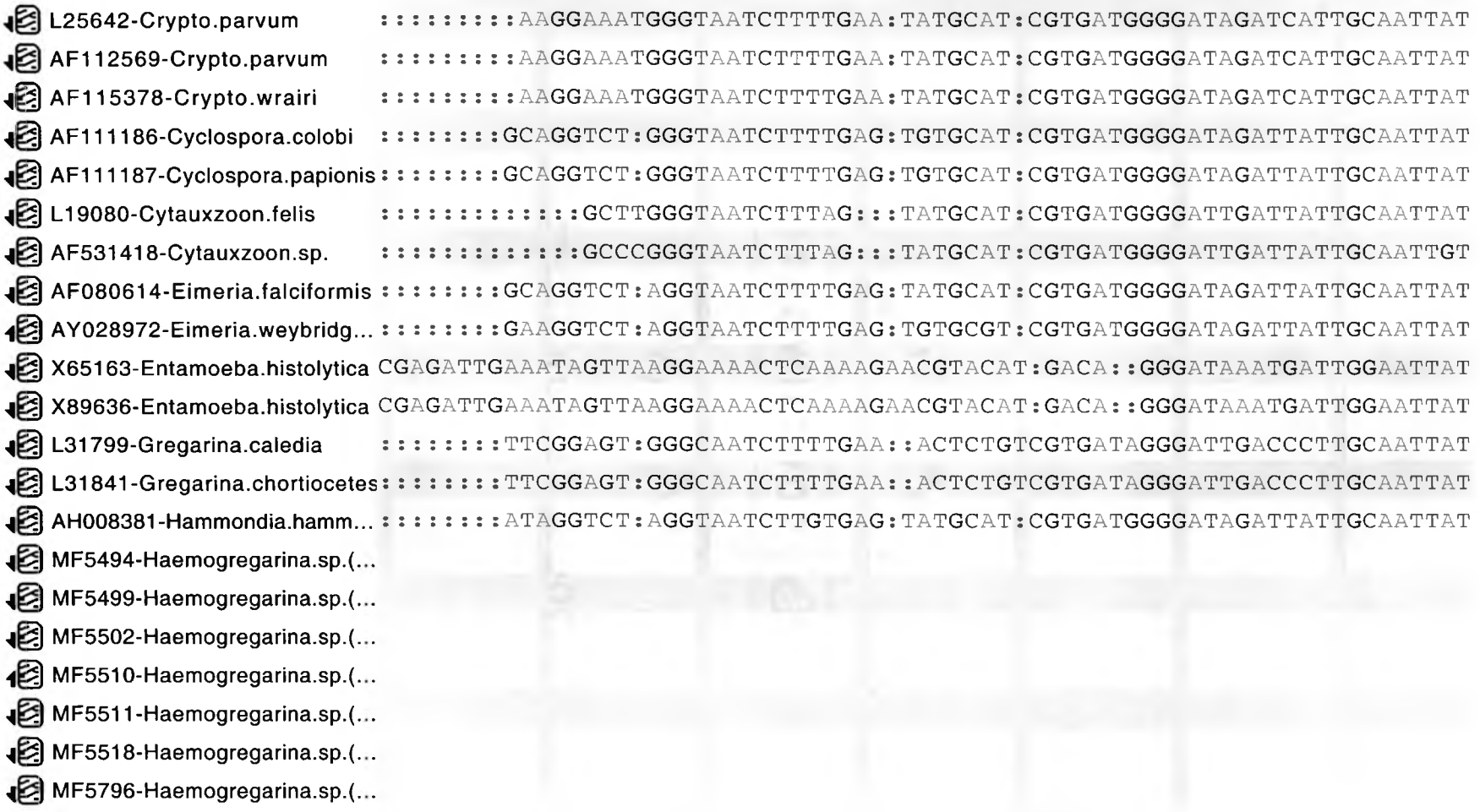
Appendix C (cont.)

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X65163-Entamoeba.histolytica TTGTTT:TGAACGAGG:AATTCCTTGTAATATCGAGTCATTAACTCGAGA:TGAATACGTCCCTGCCCT
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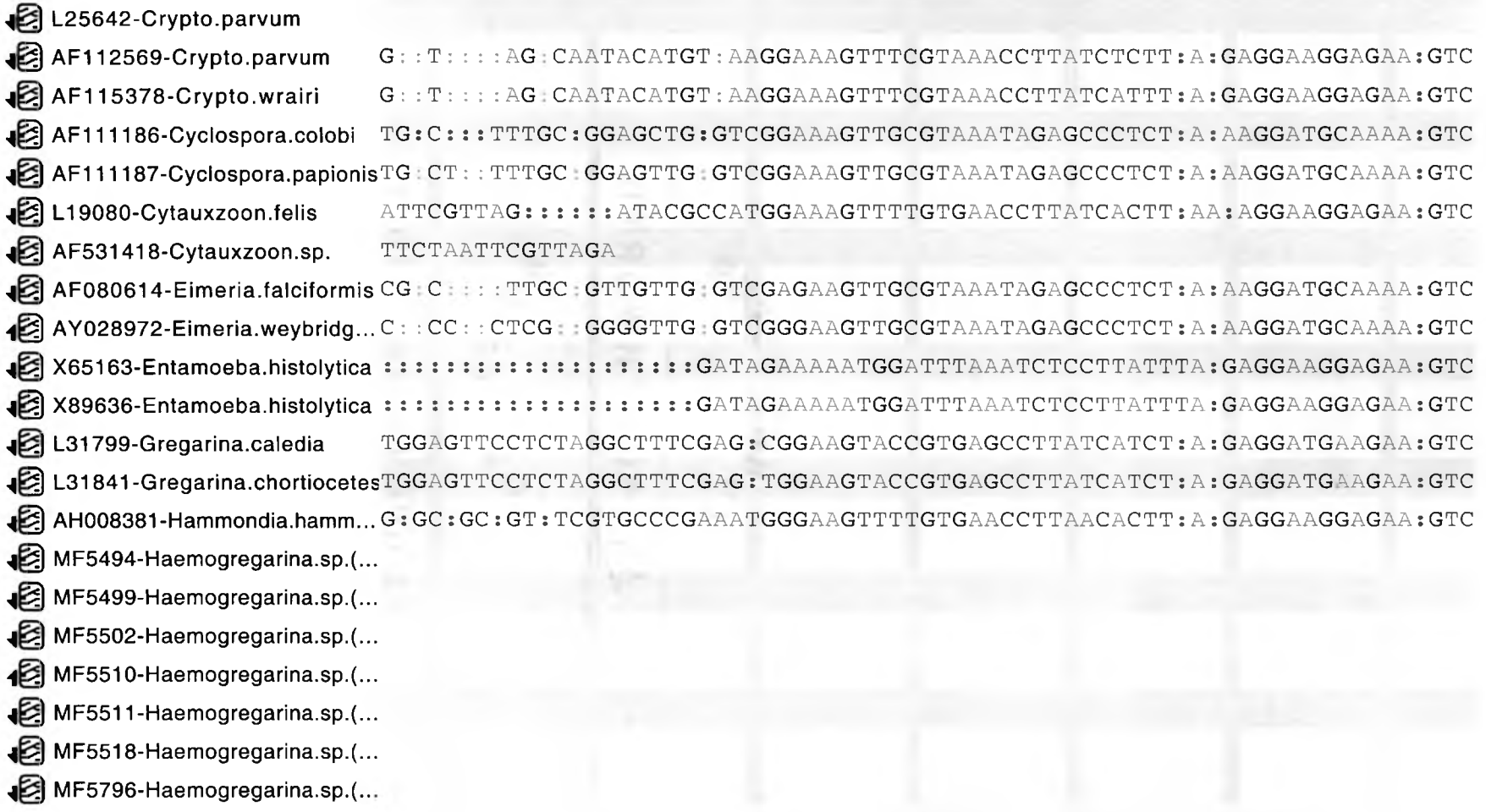
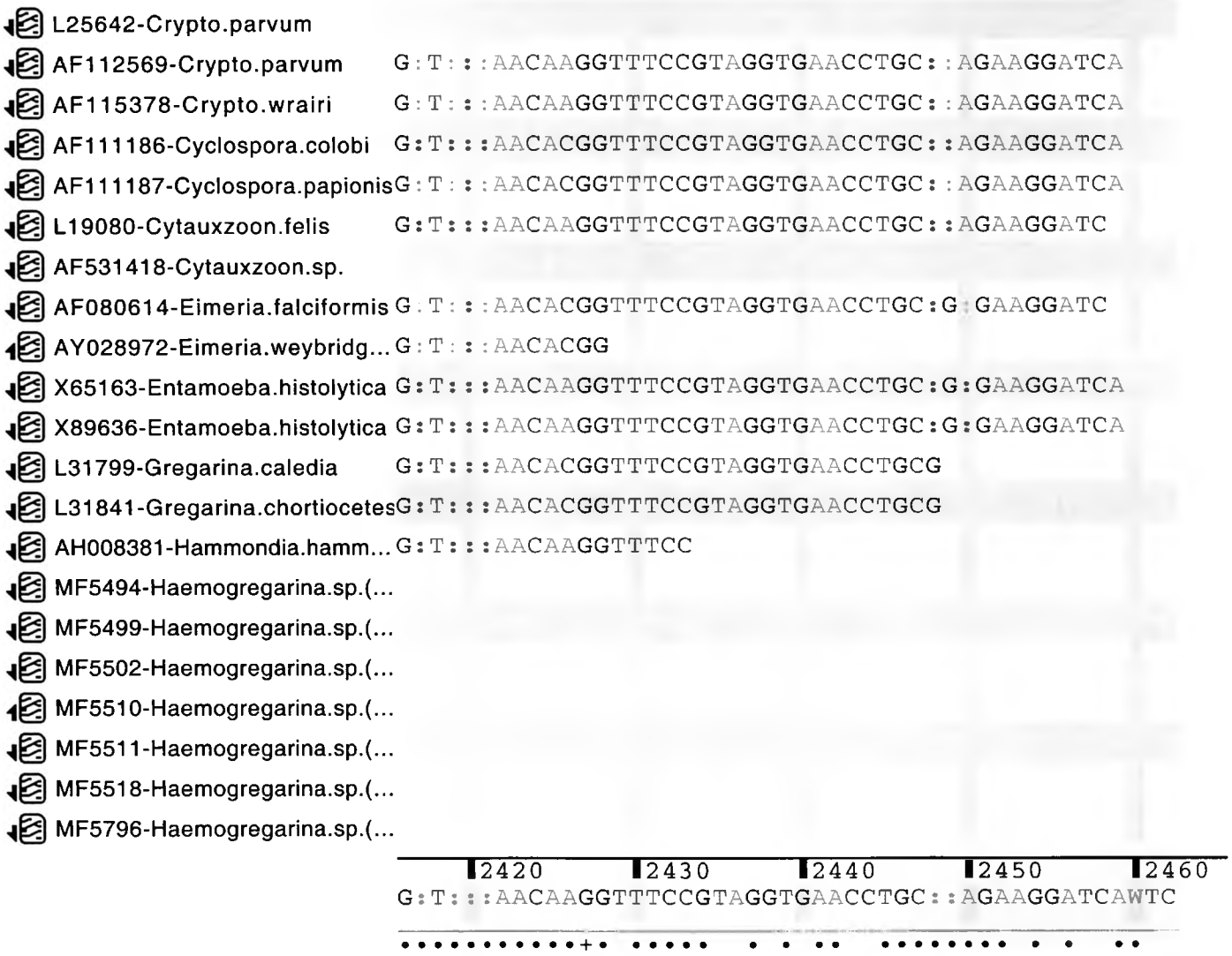
Appendix C (cont.)

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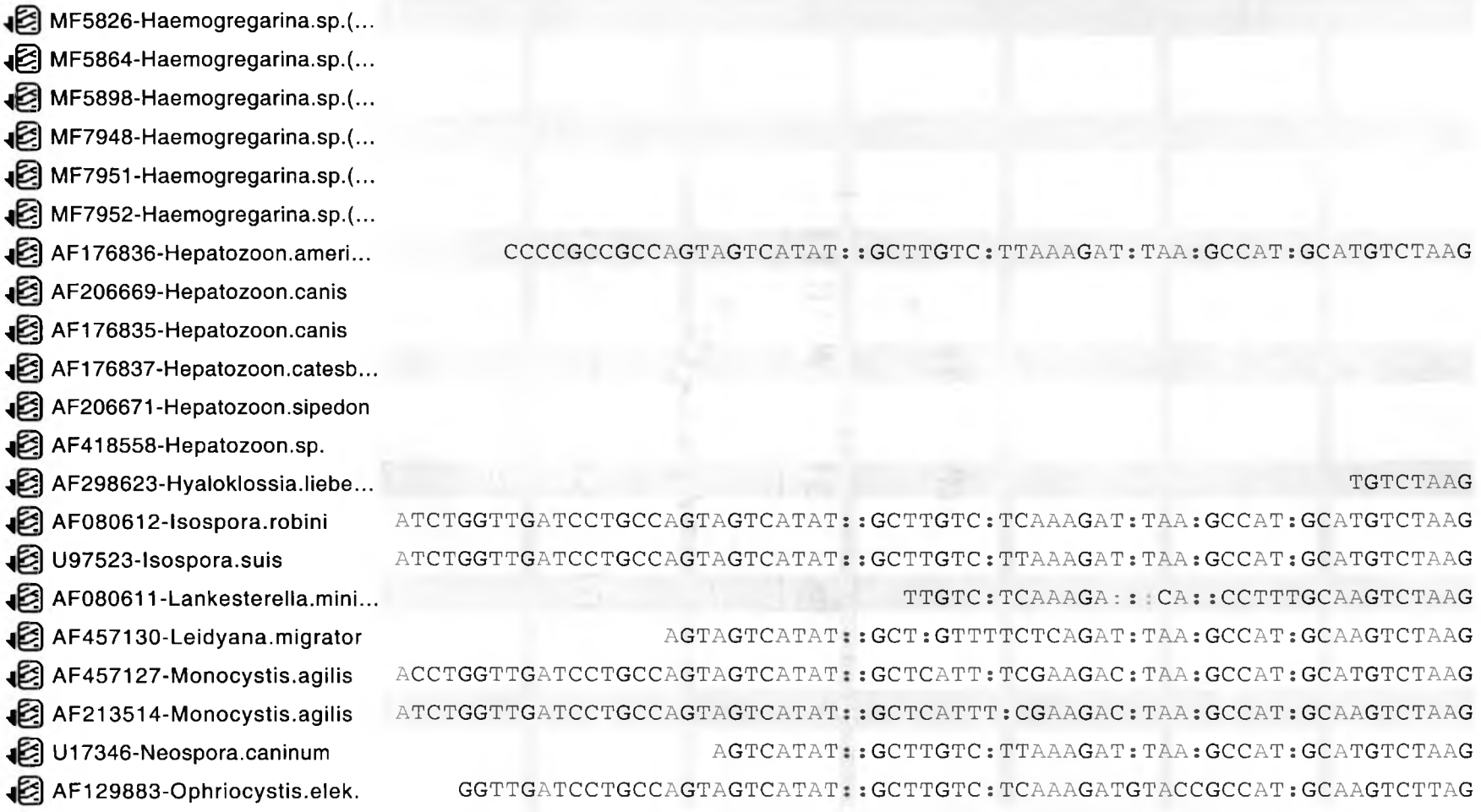
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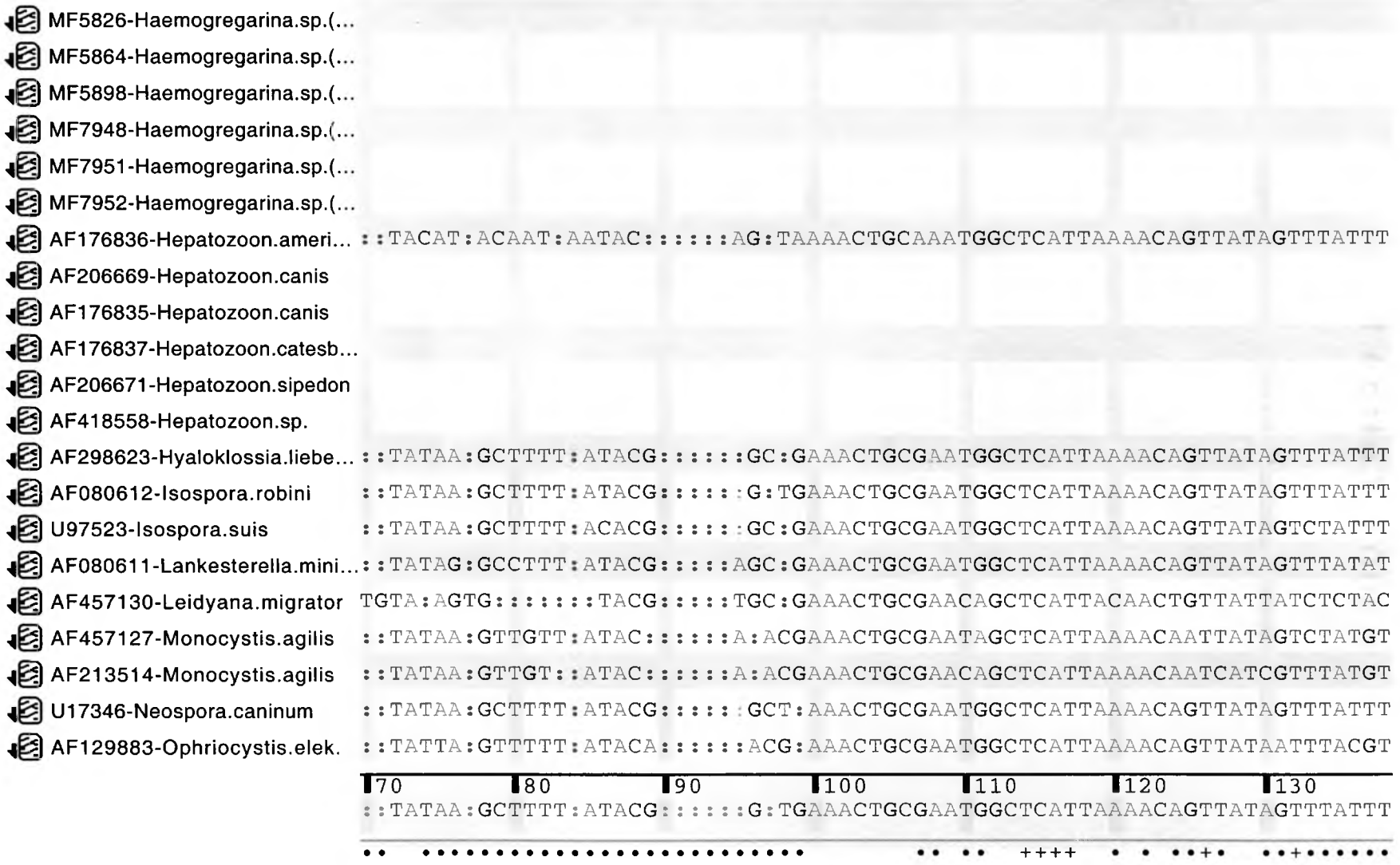
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Appendix C (cont.)


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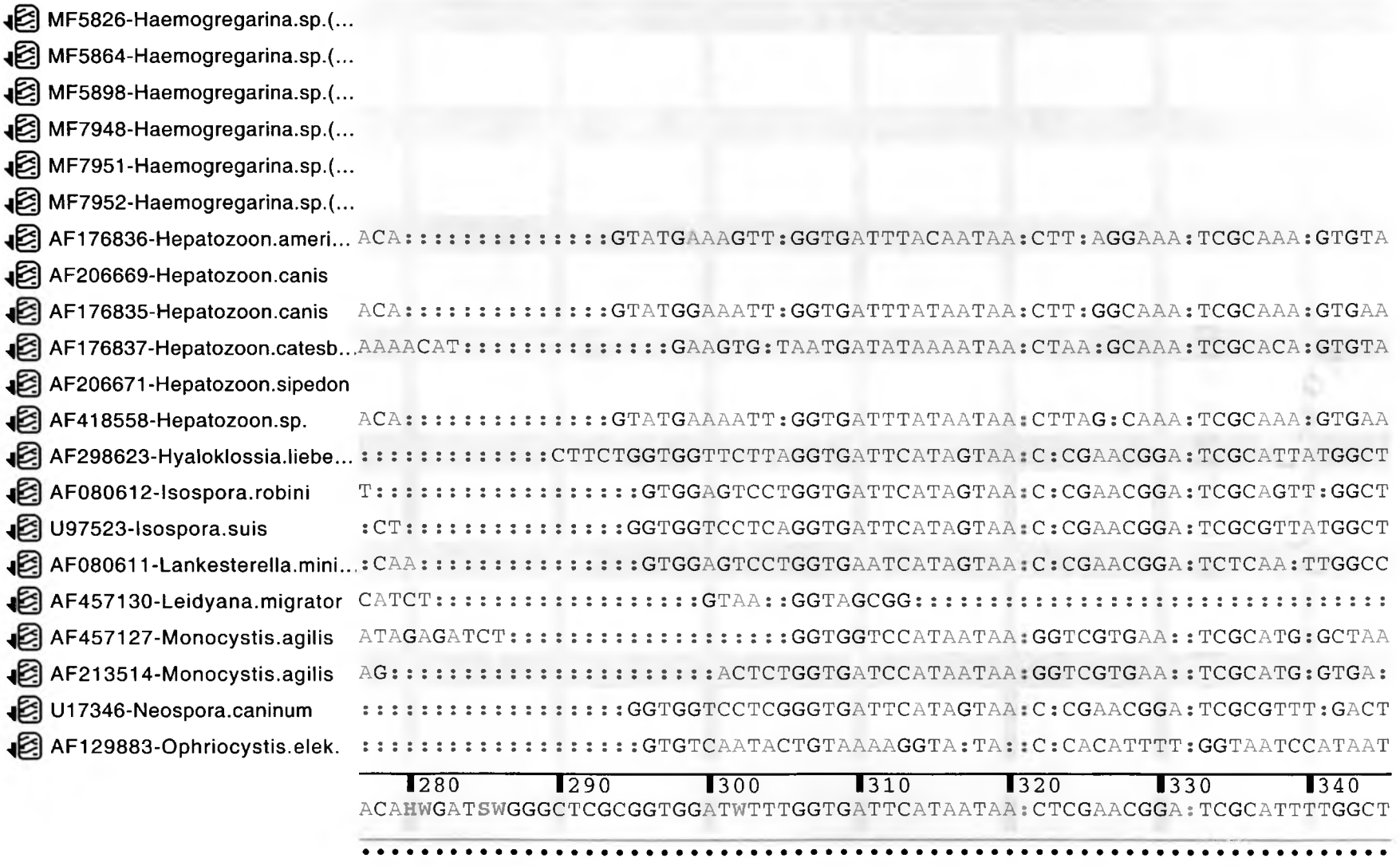

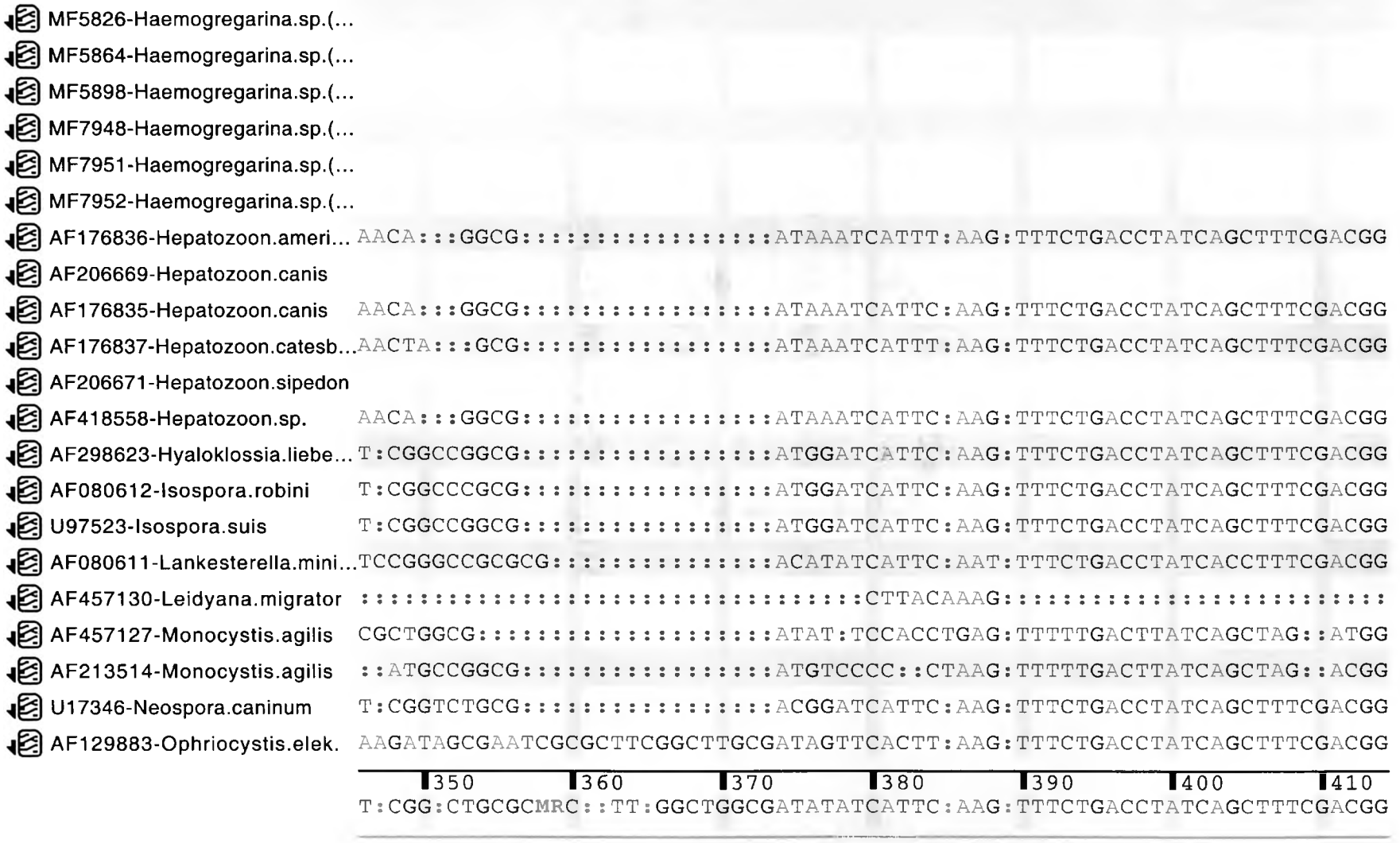
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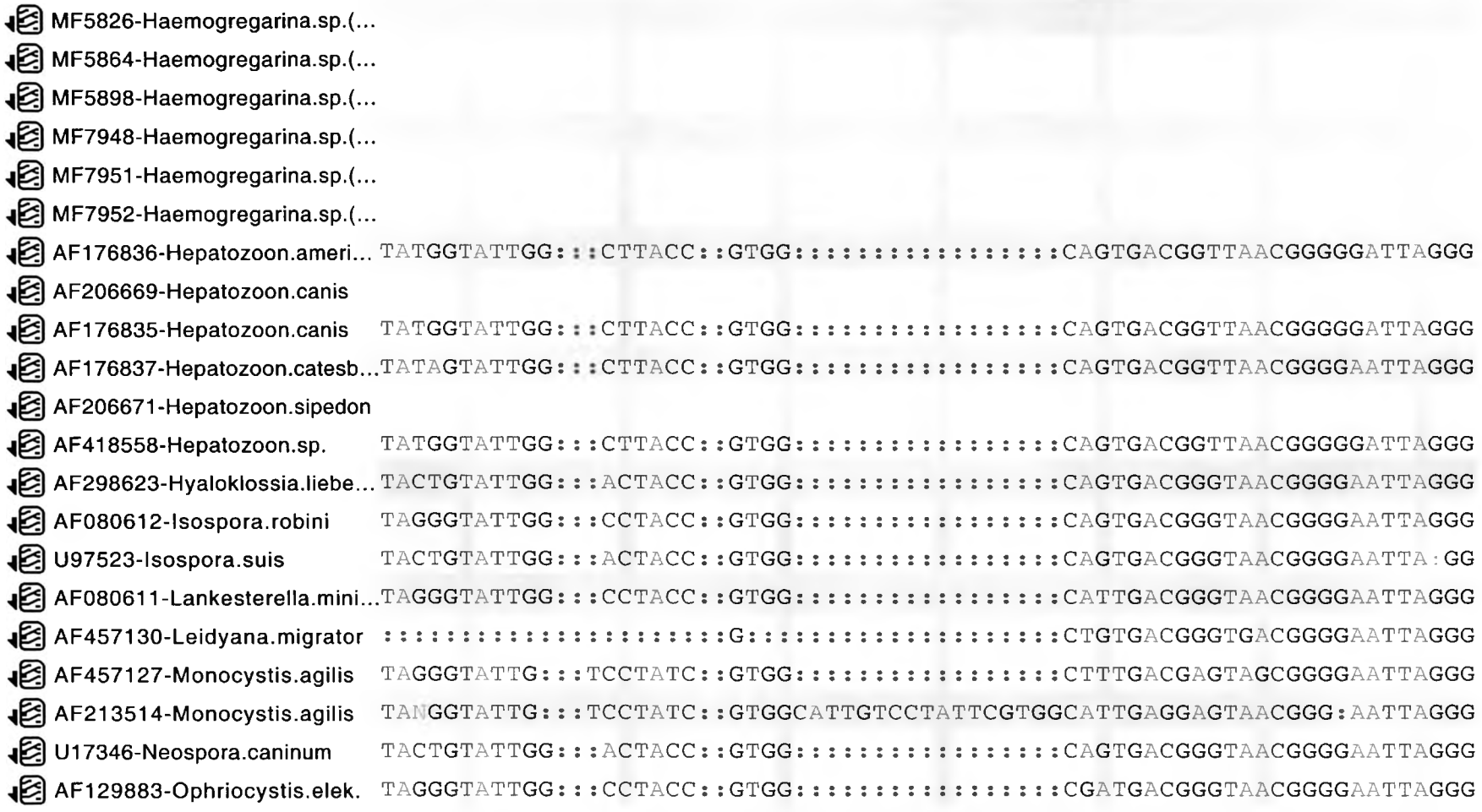
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U17346-Neospora.caninum TACTGTATTGG:::ACTACC::GTGG:::::::::::::::::CAGTGACGGGTAACGGGGAATTAGGG
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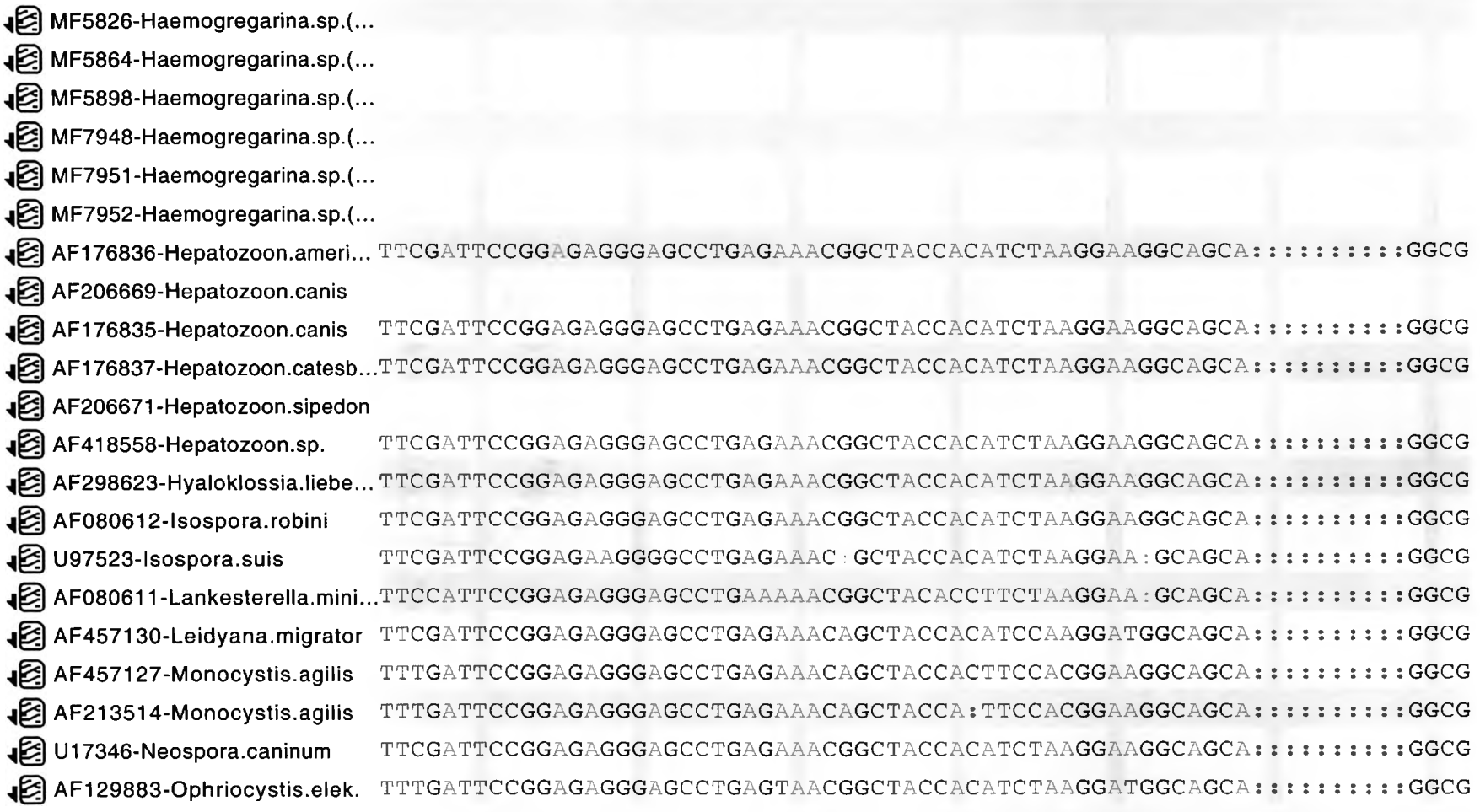
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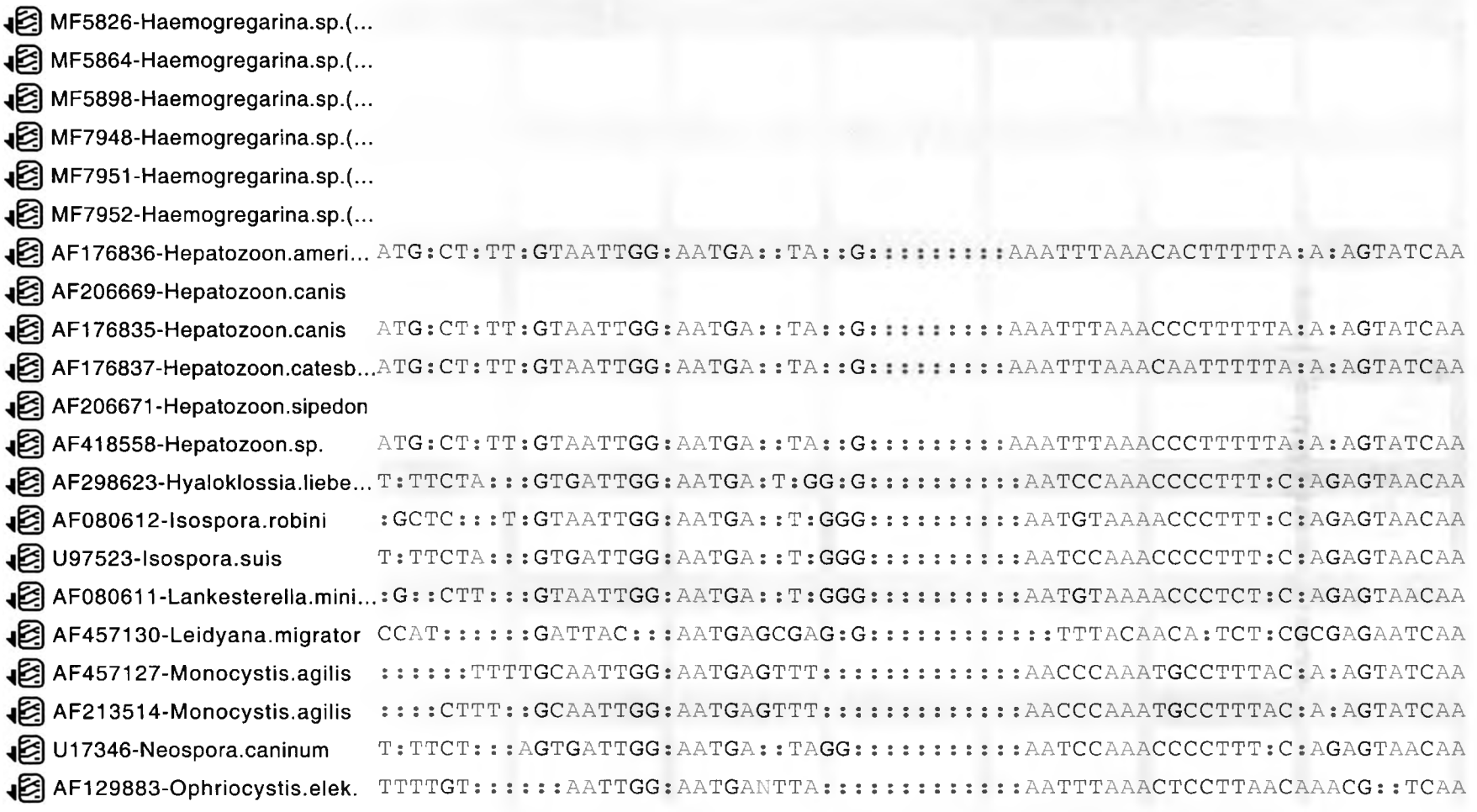
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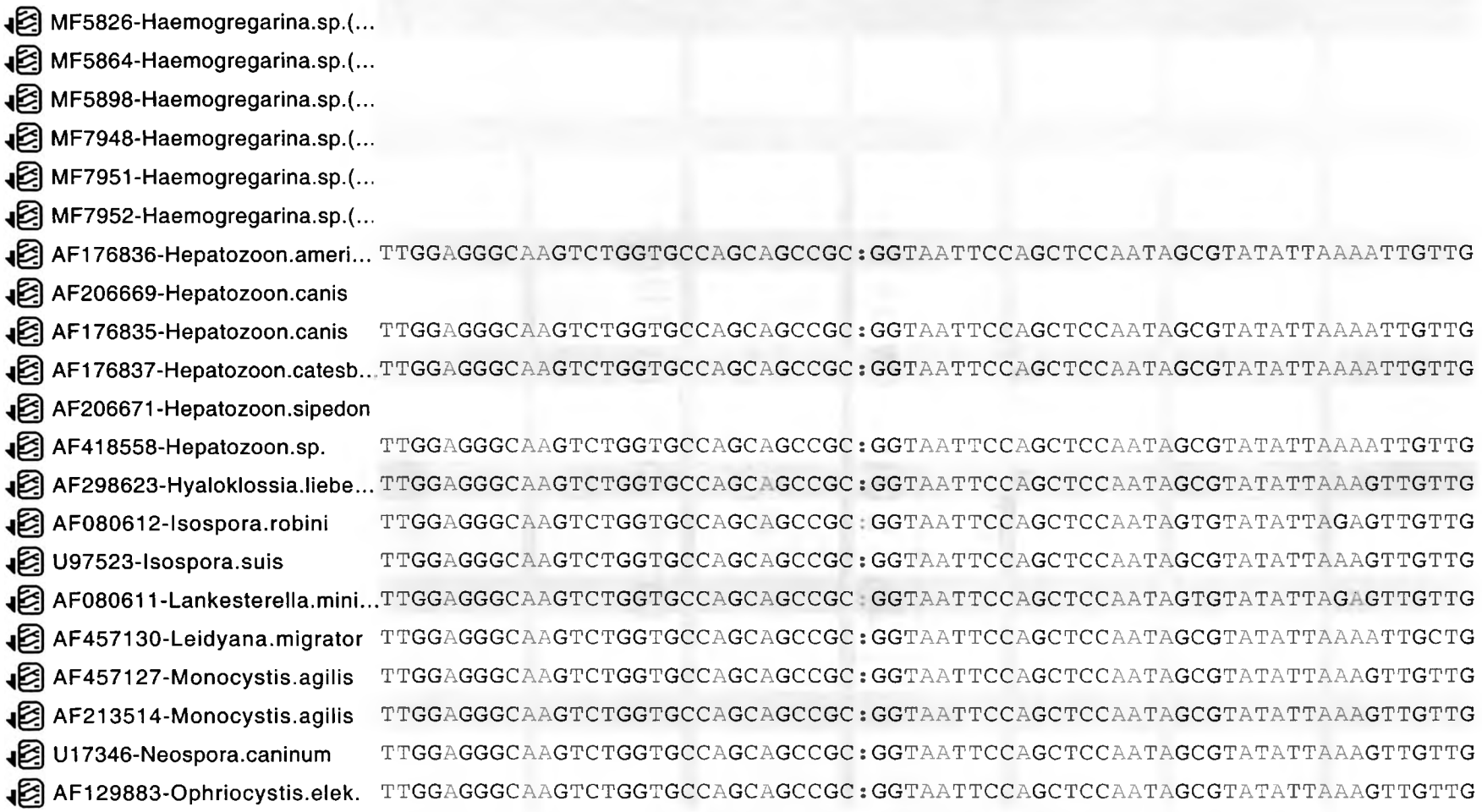
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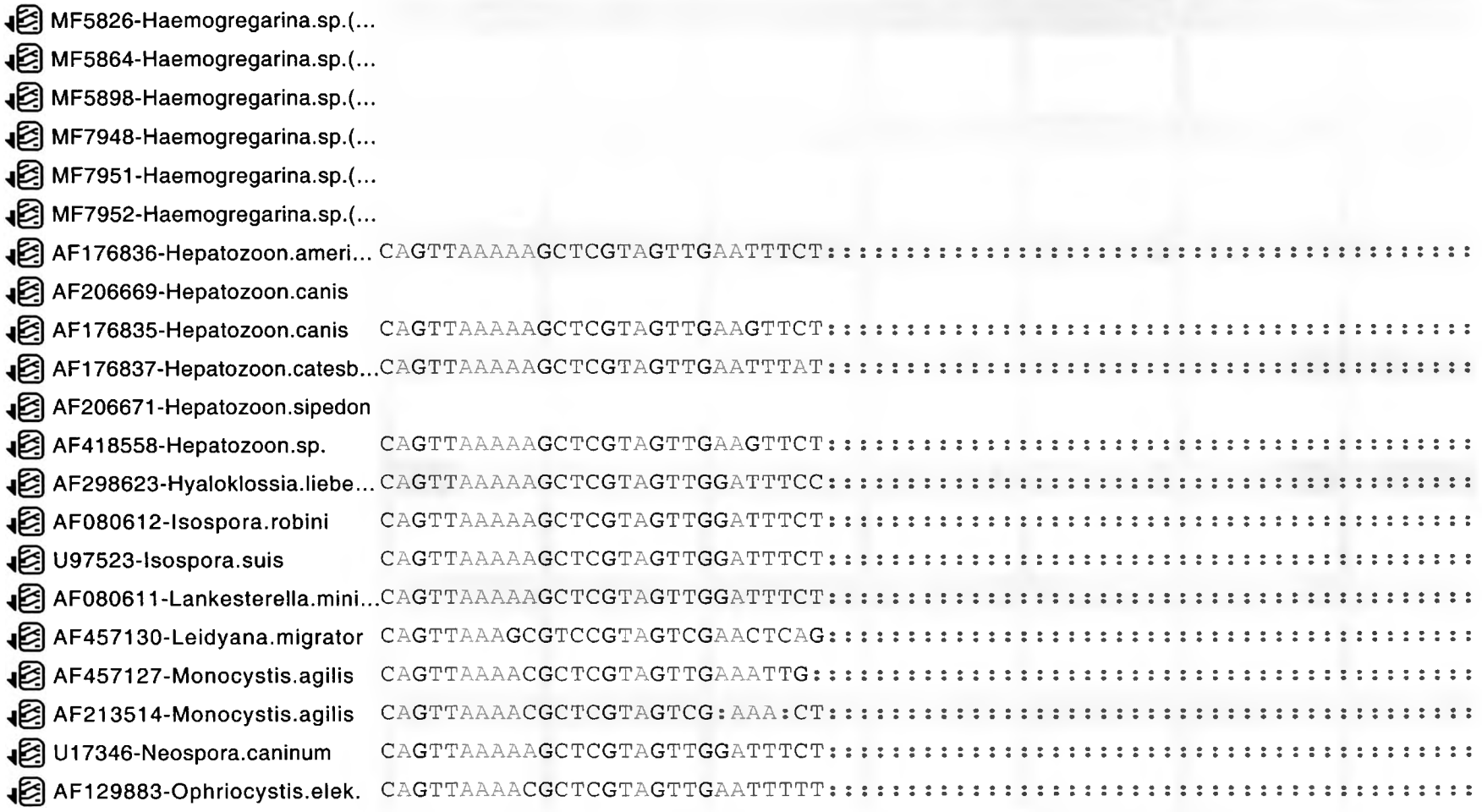
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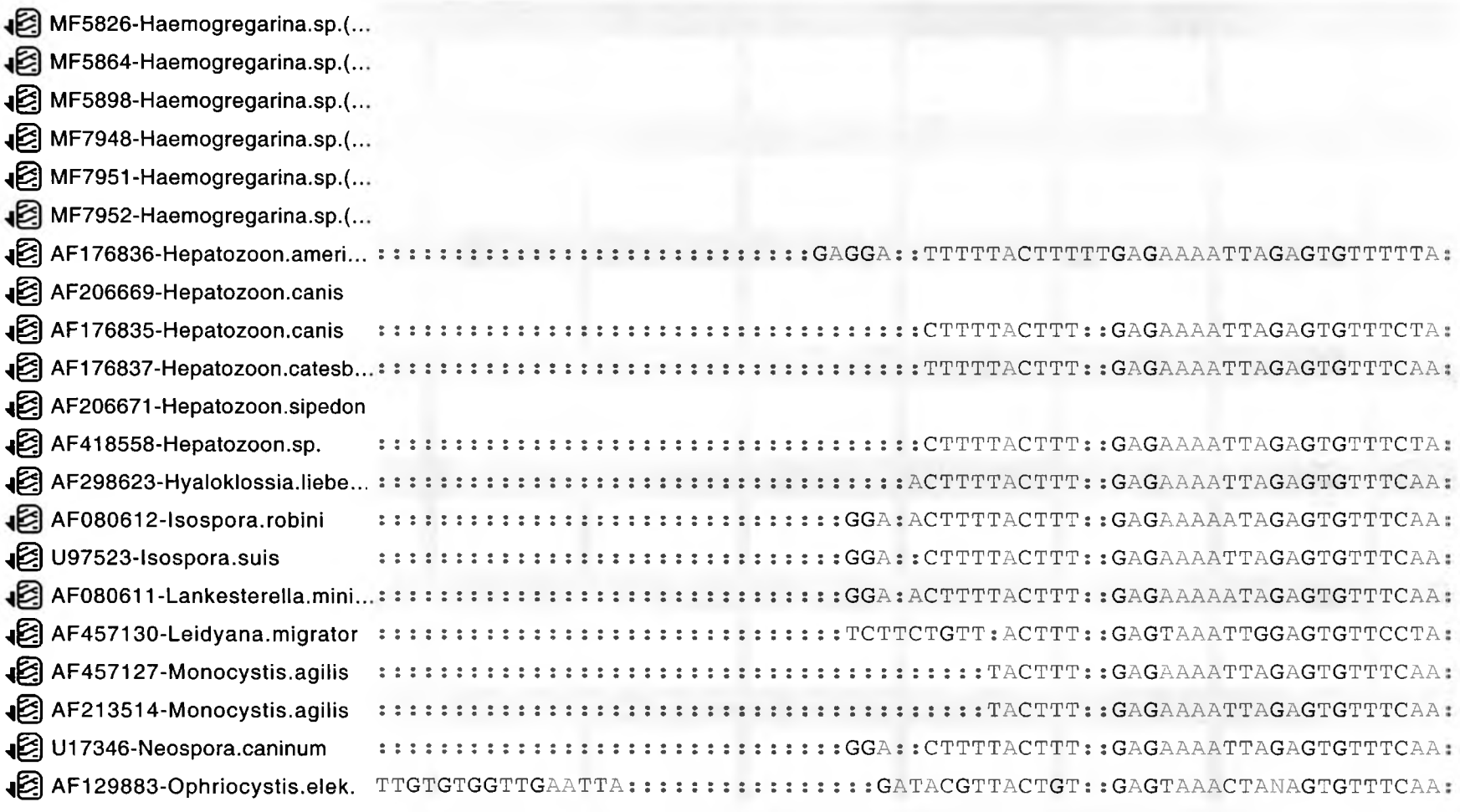
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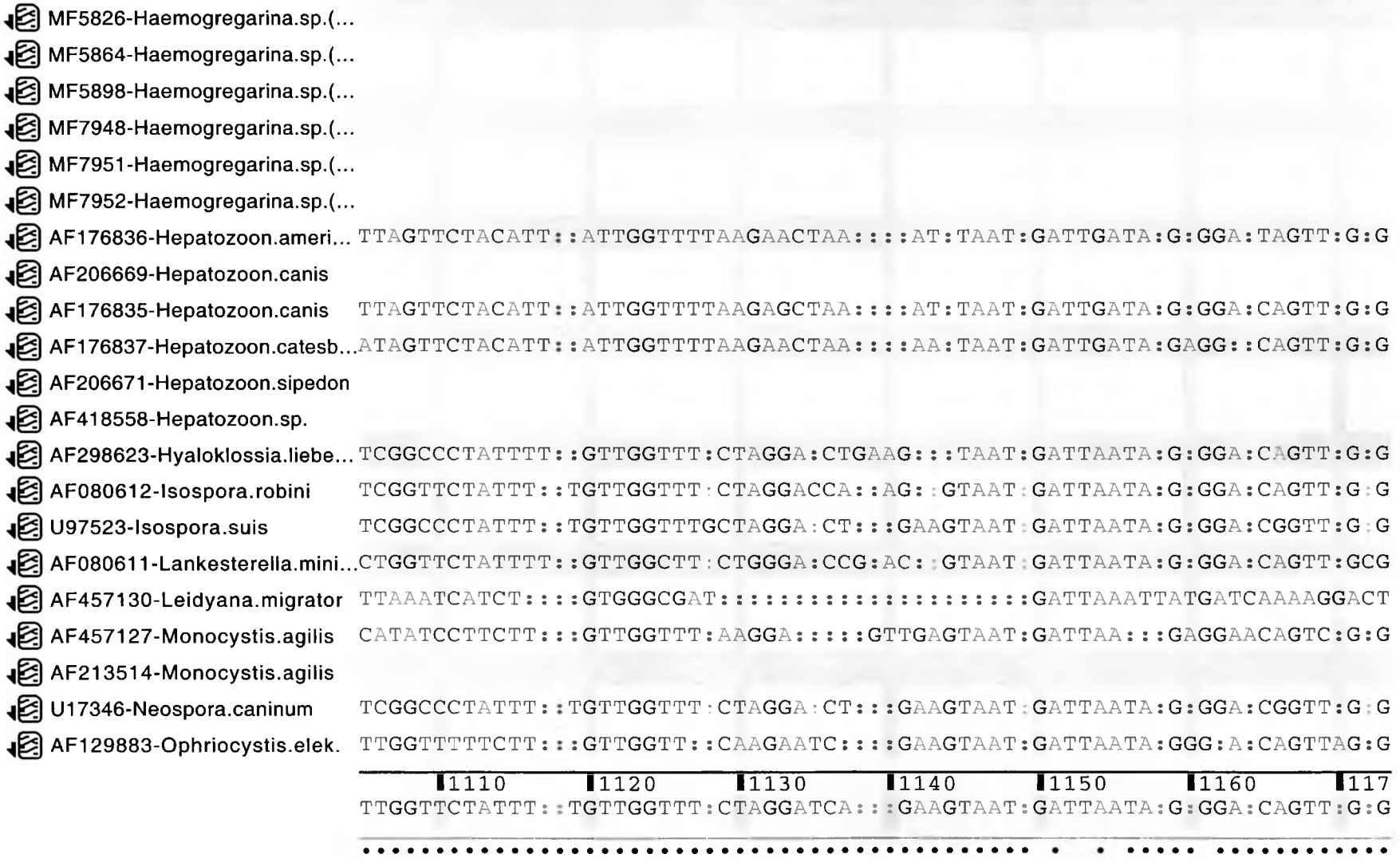
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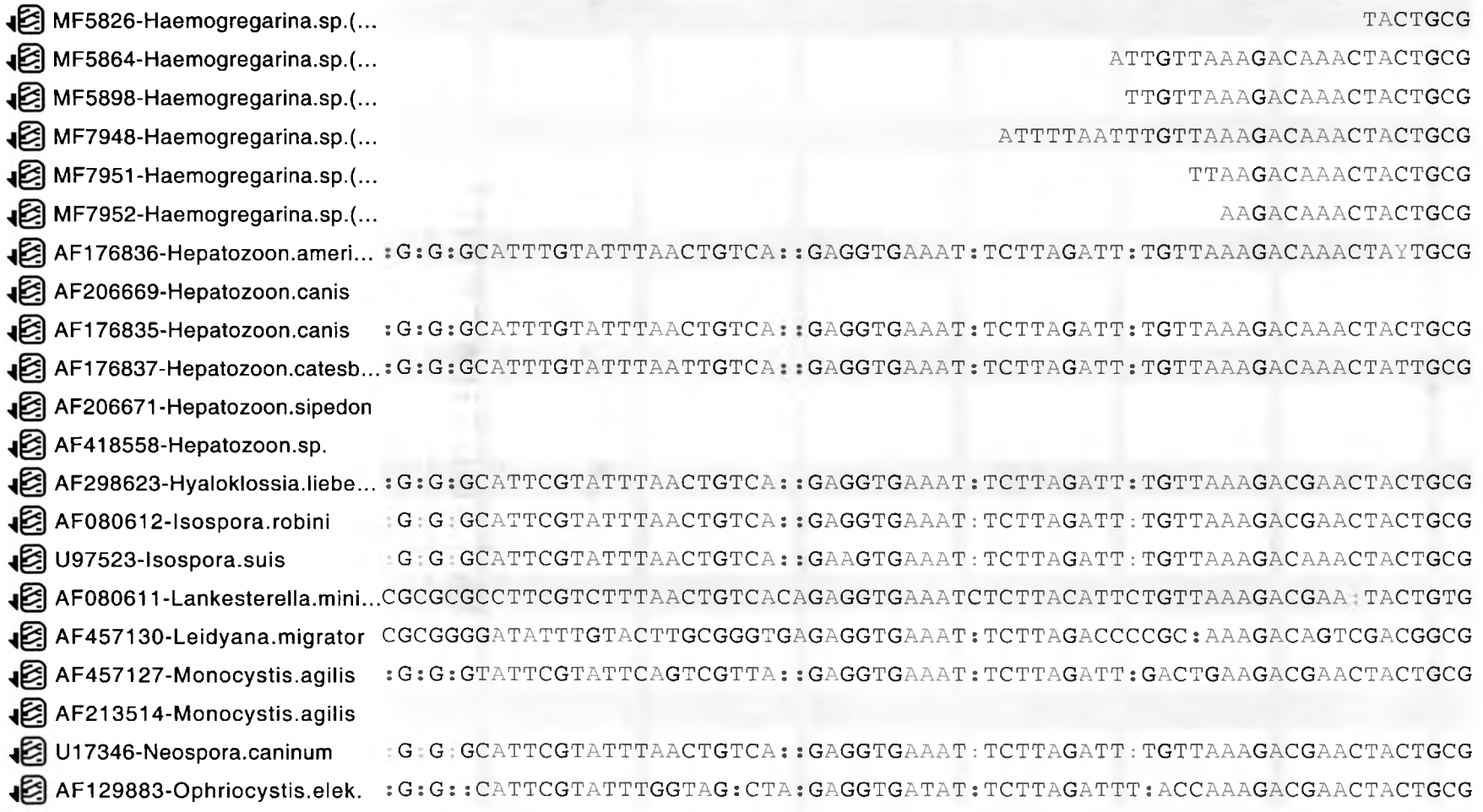
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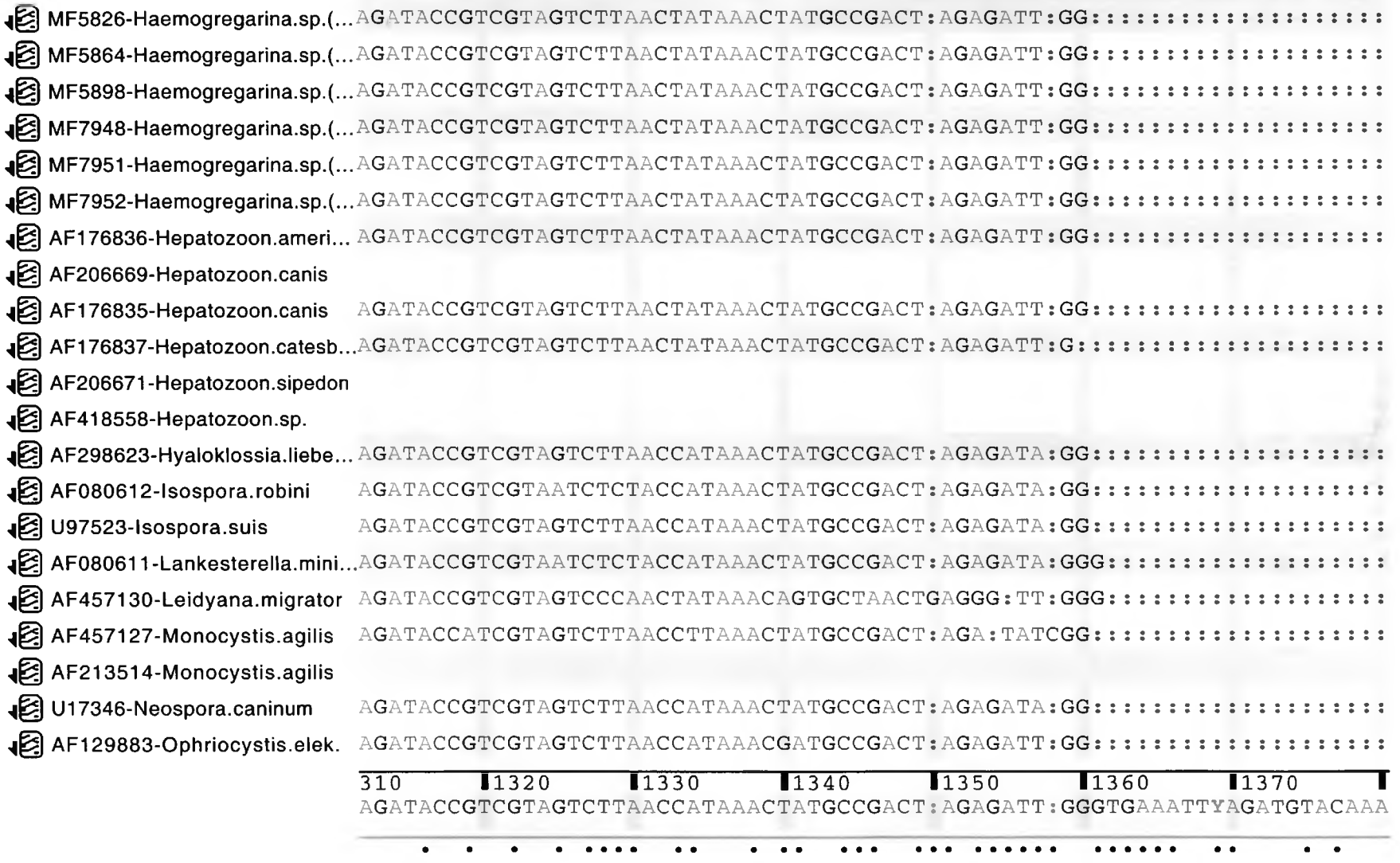
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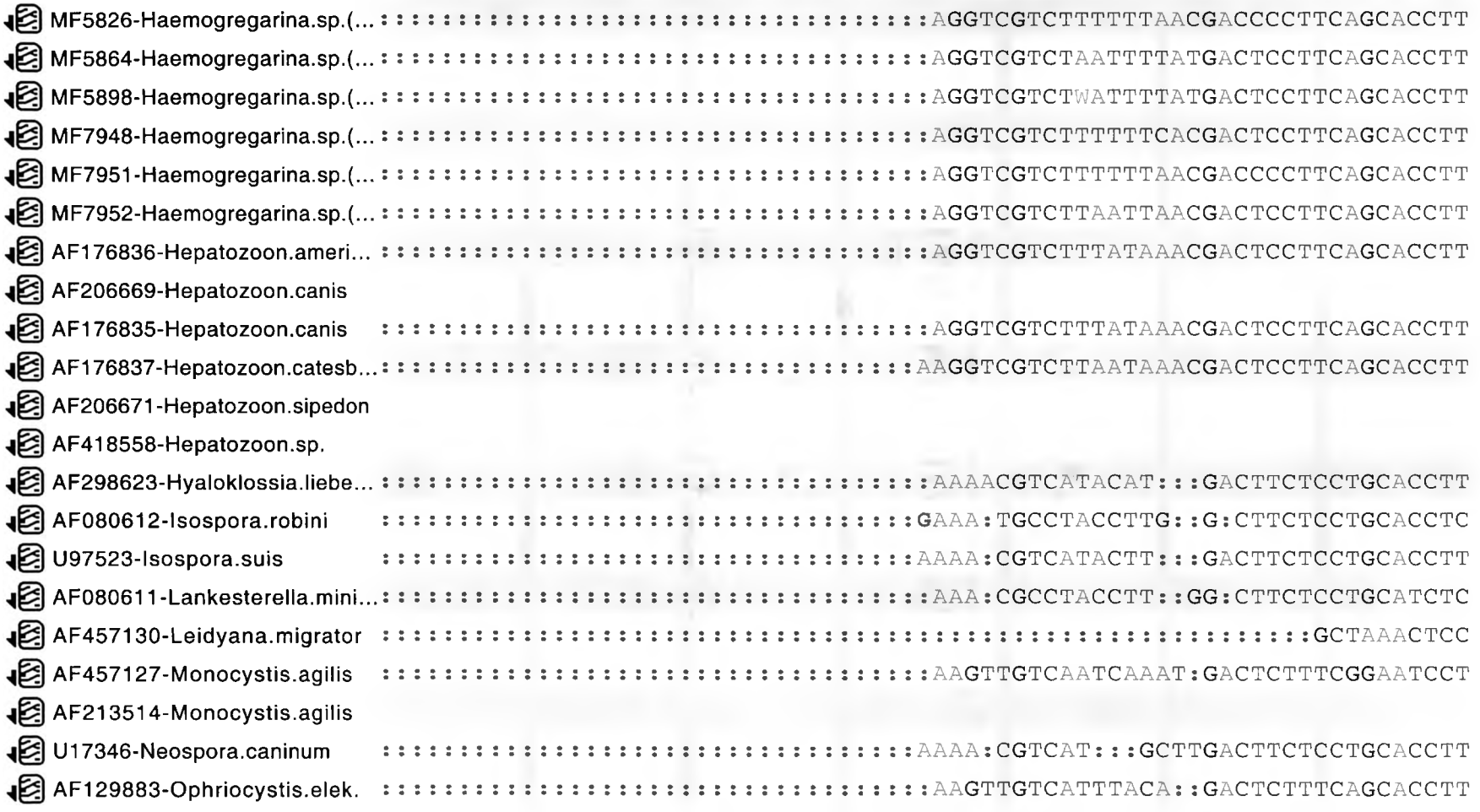
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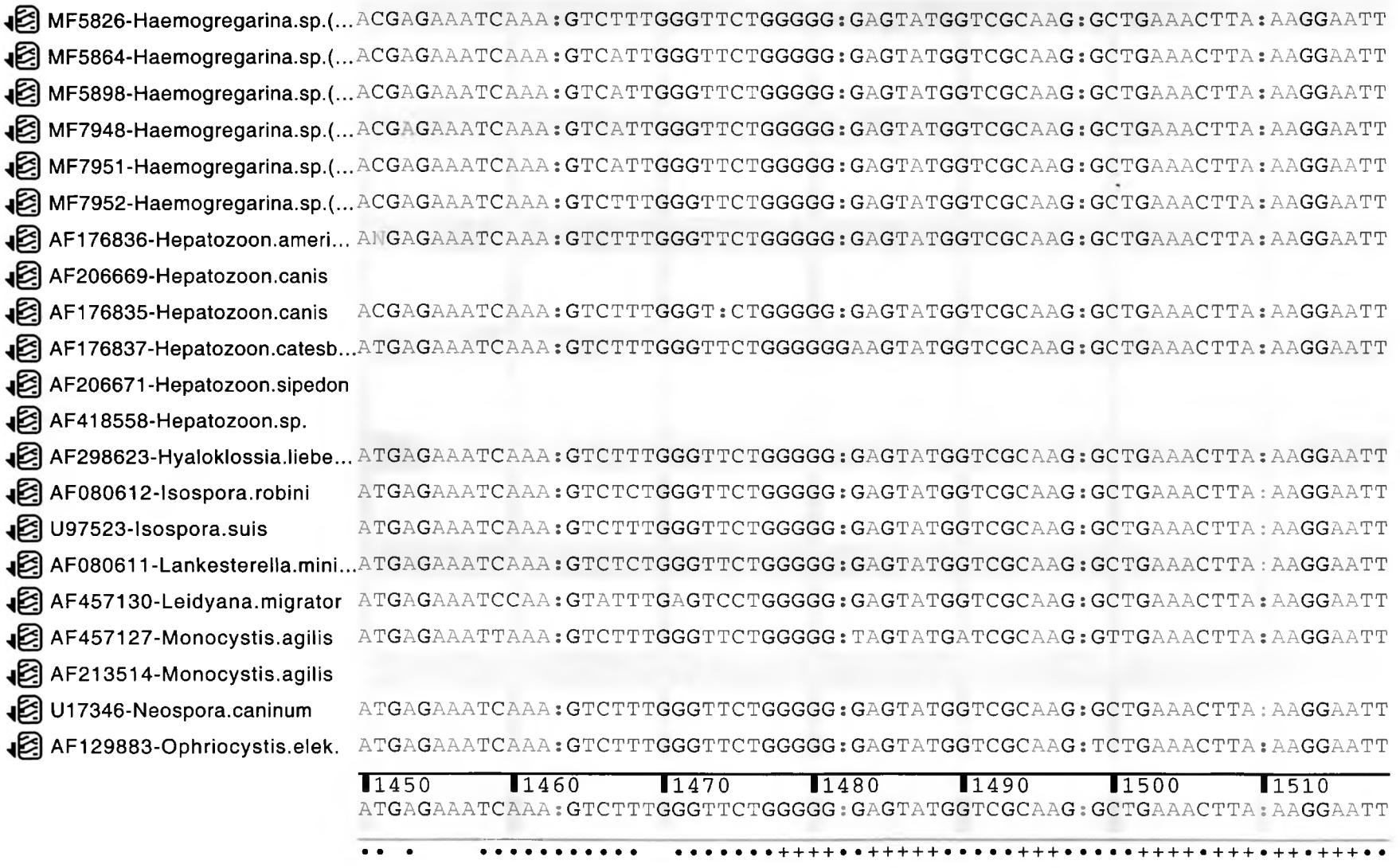
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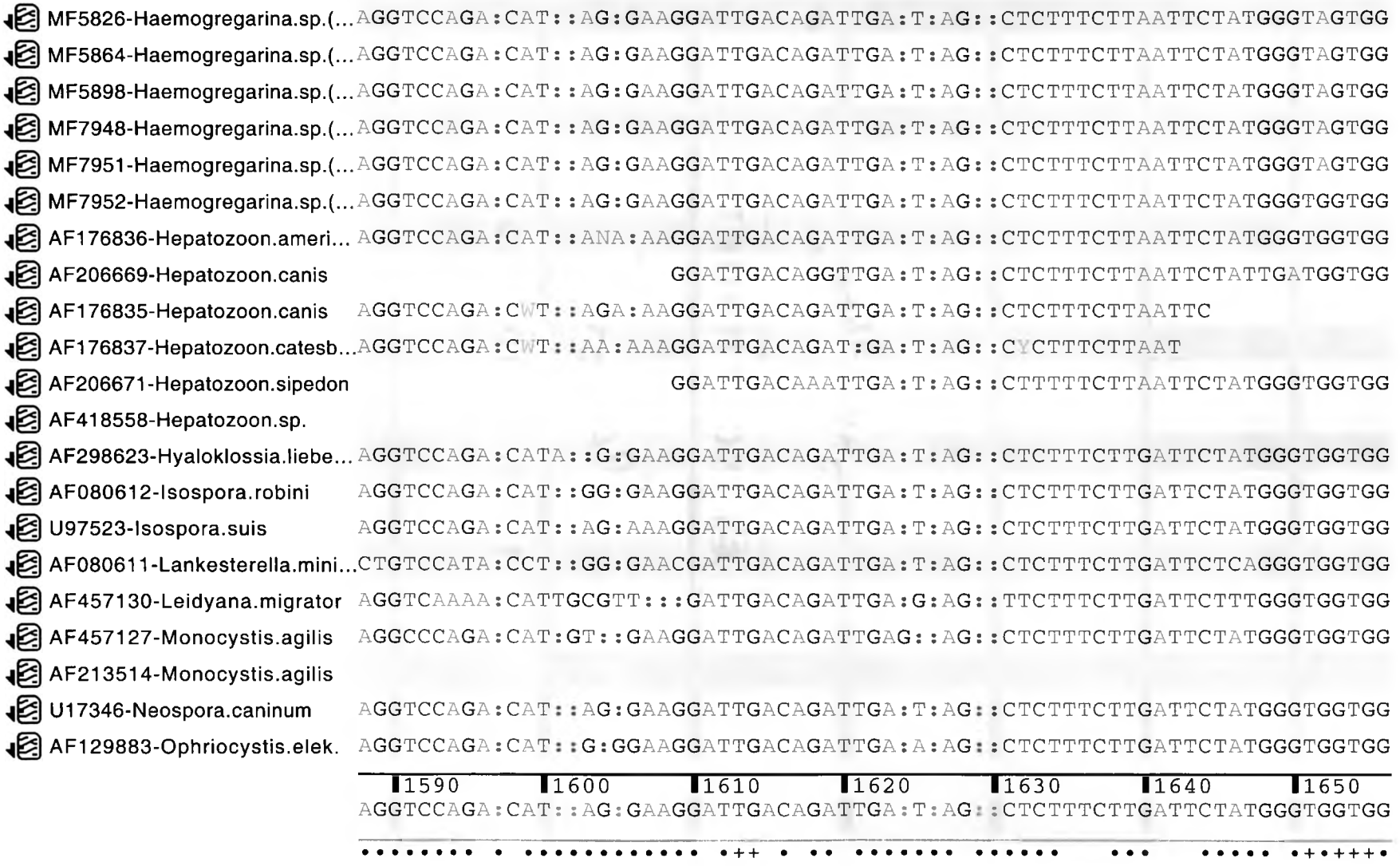
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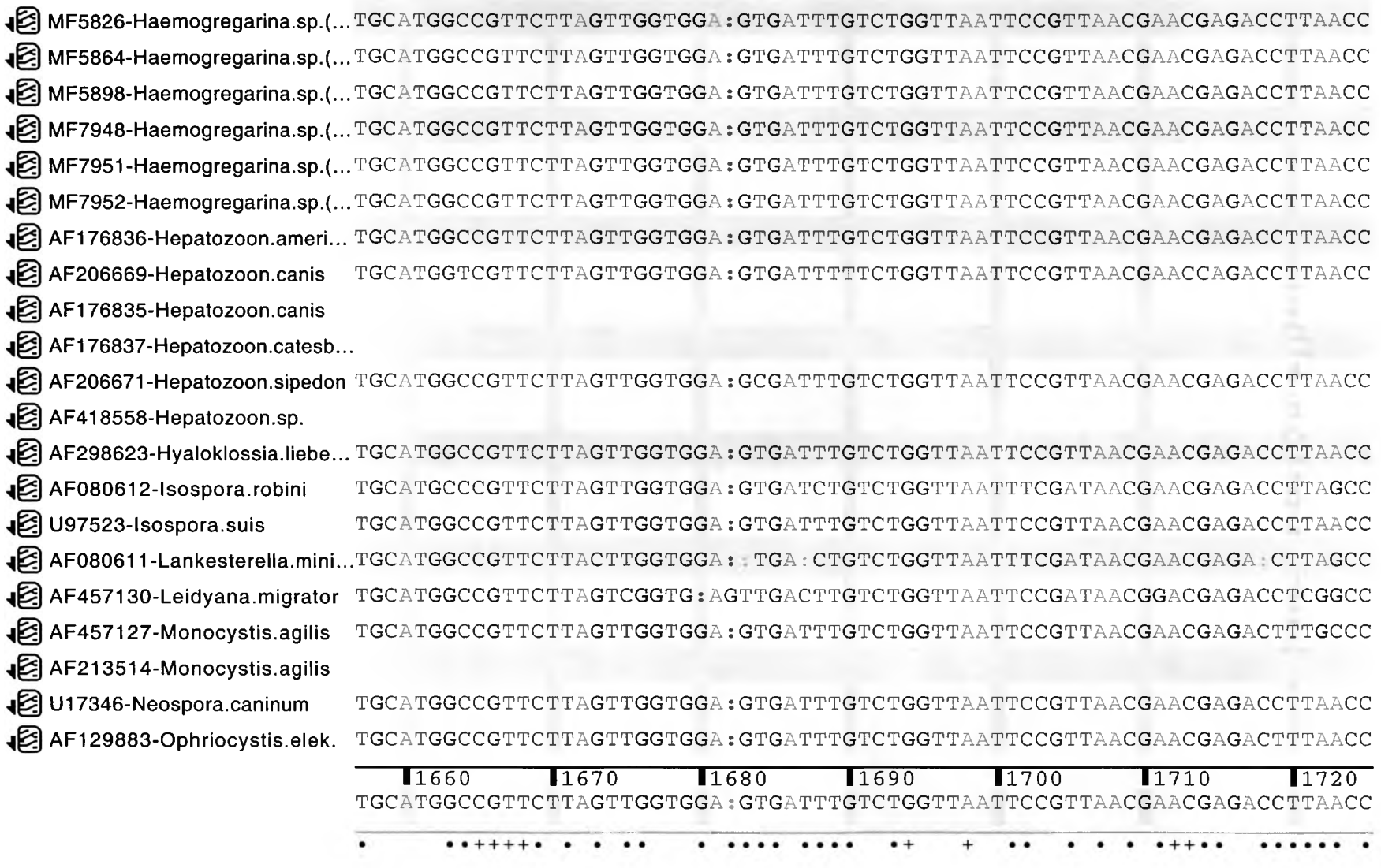
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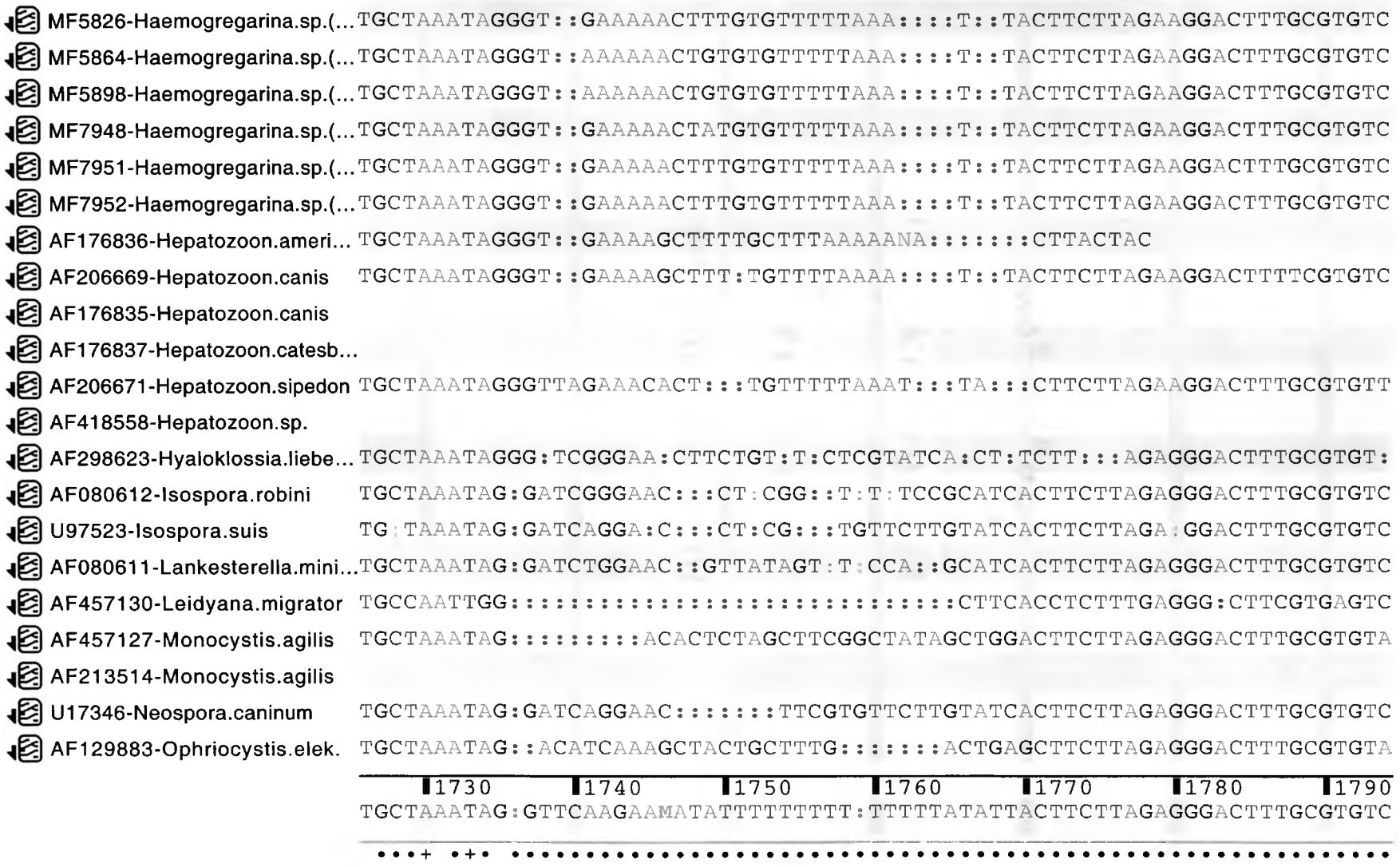

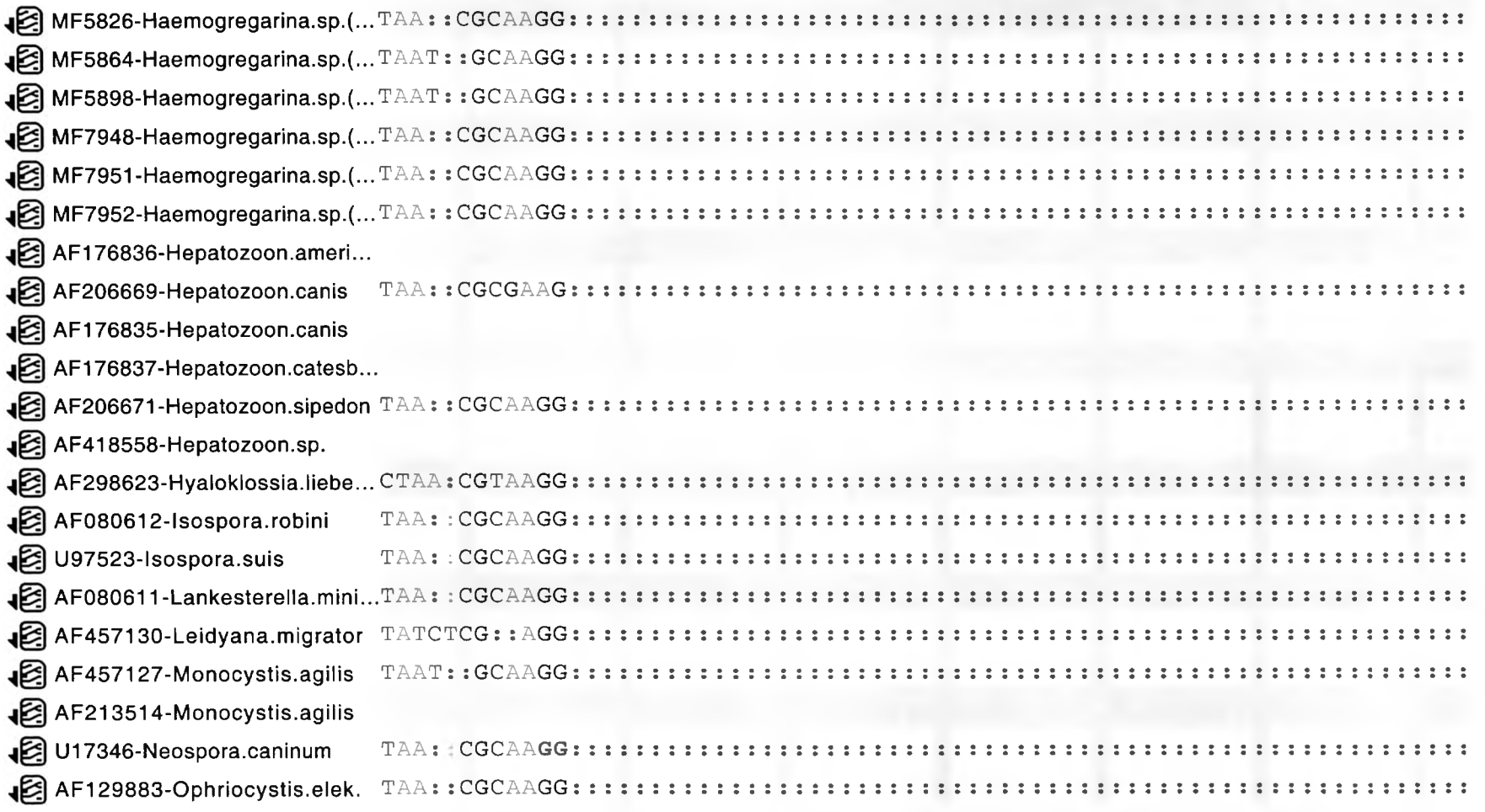
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AF457127-Monocystis.agilis
AF213514-Monocystis.agilis
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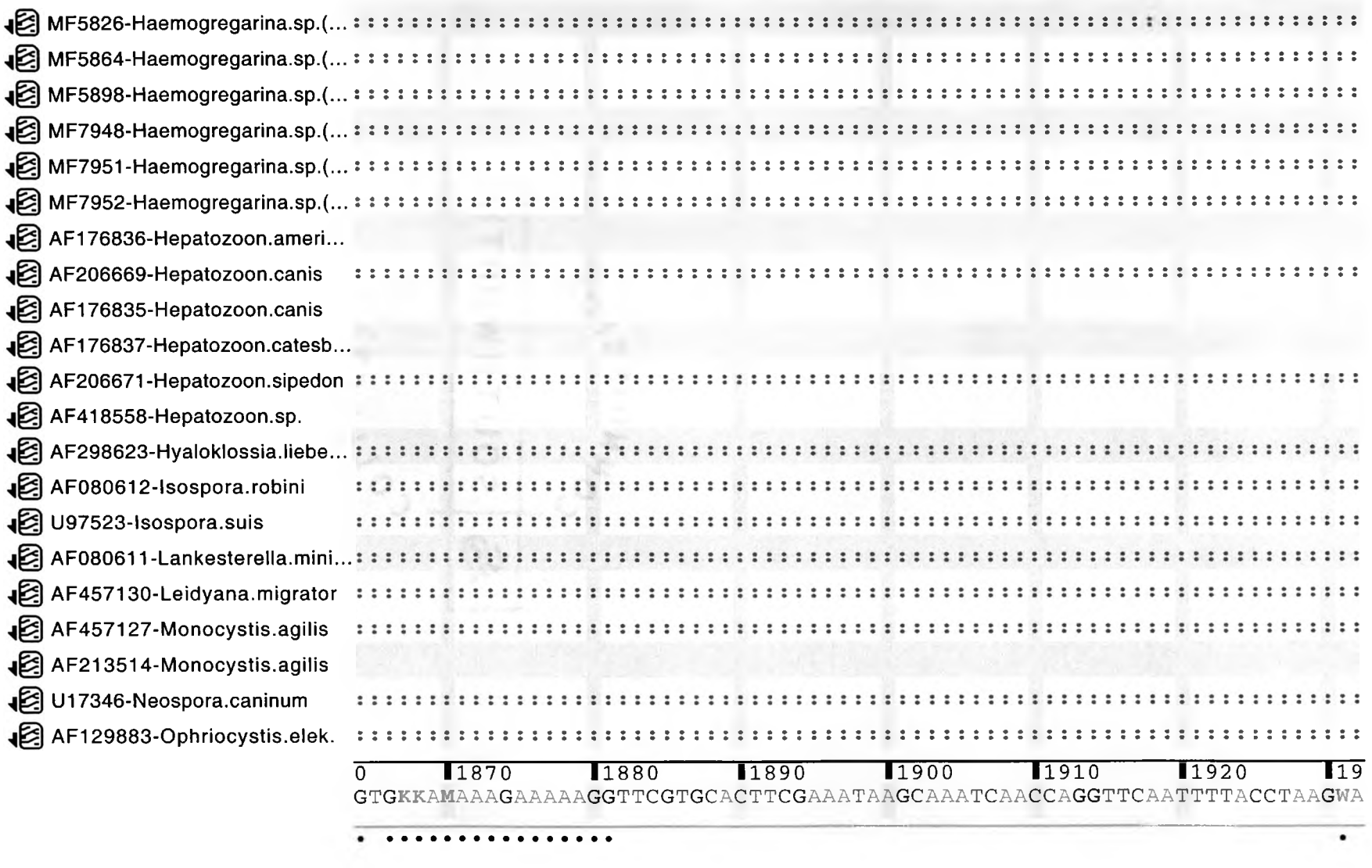
Appendix C (cont.)



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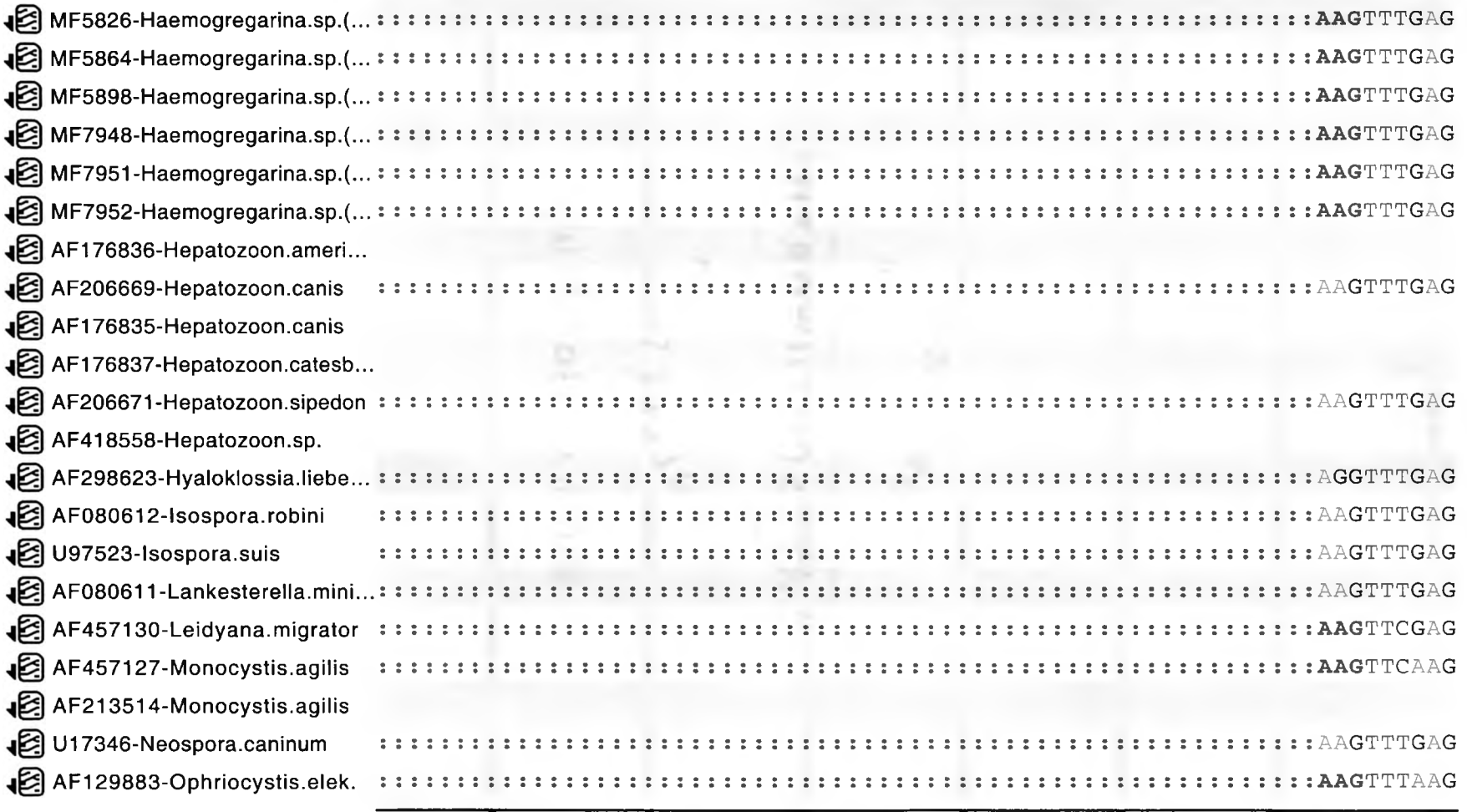


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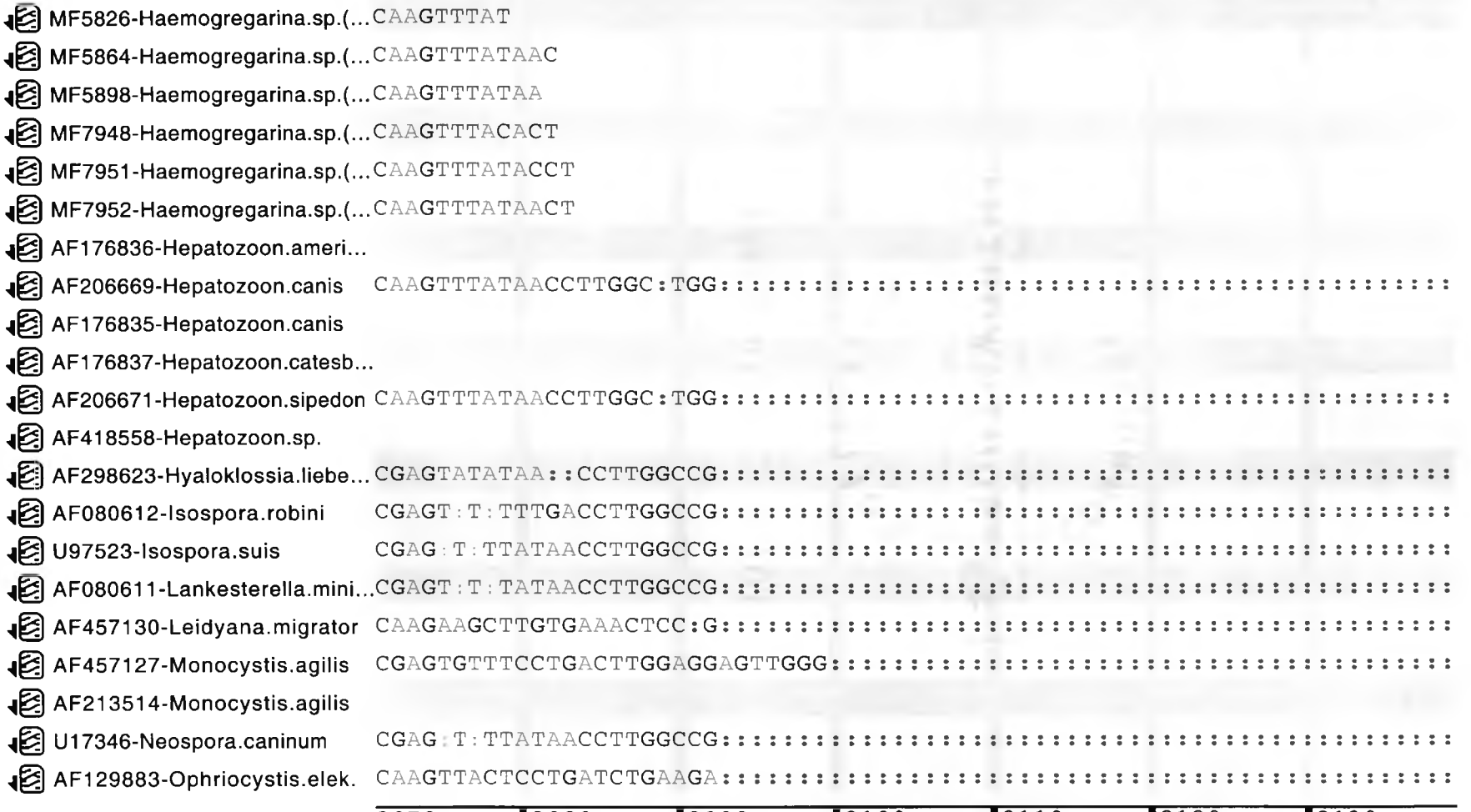
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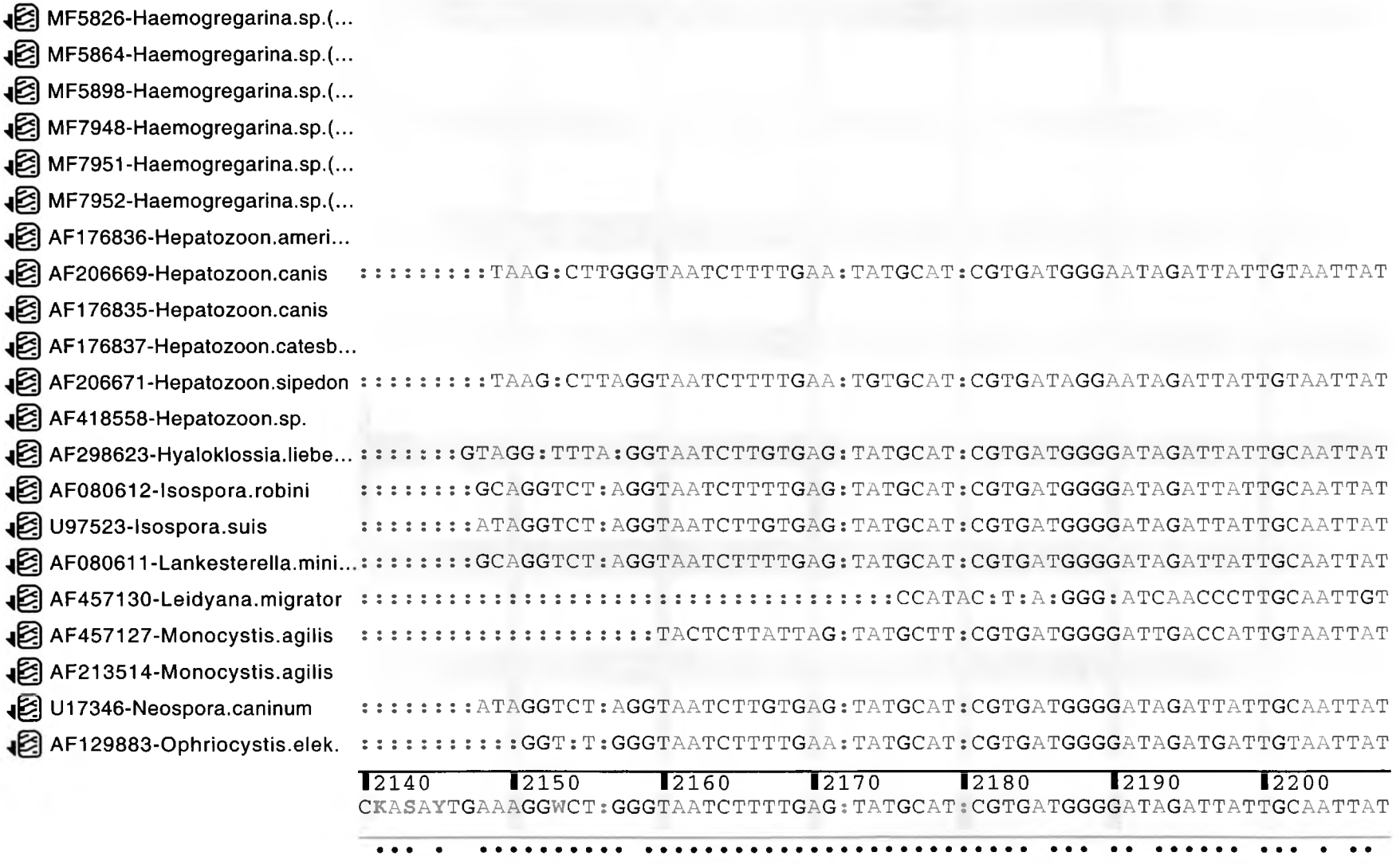
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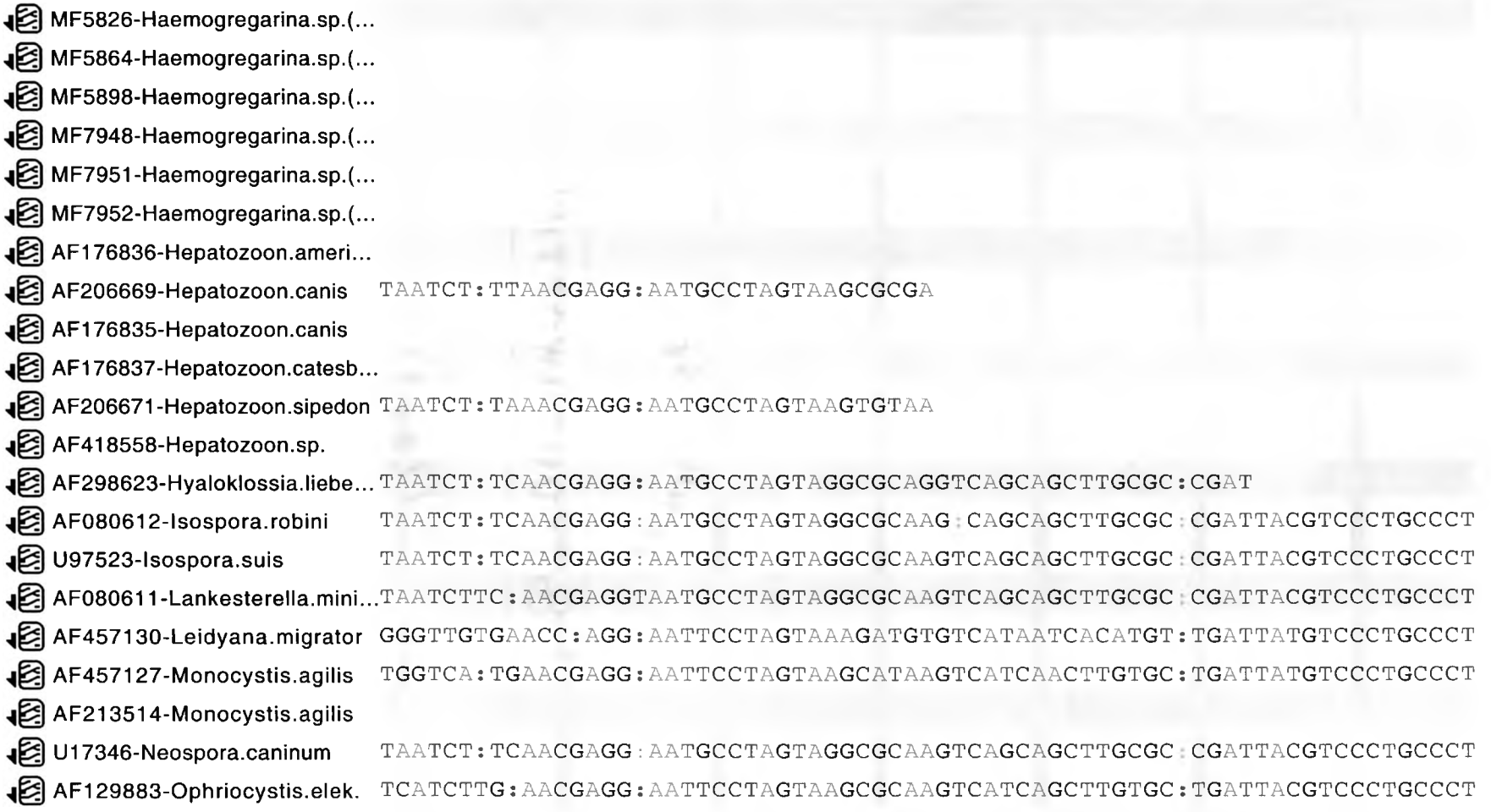
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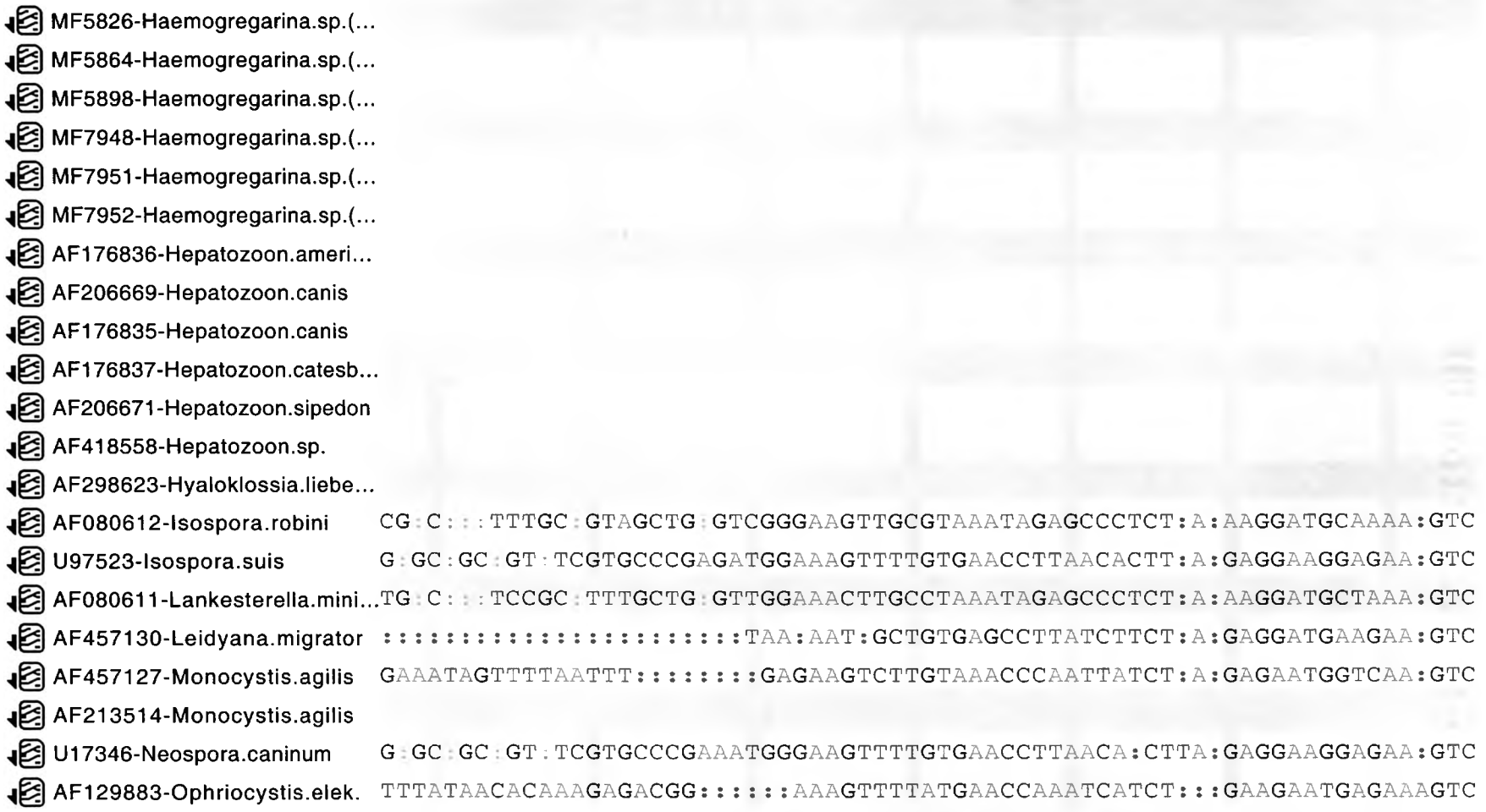
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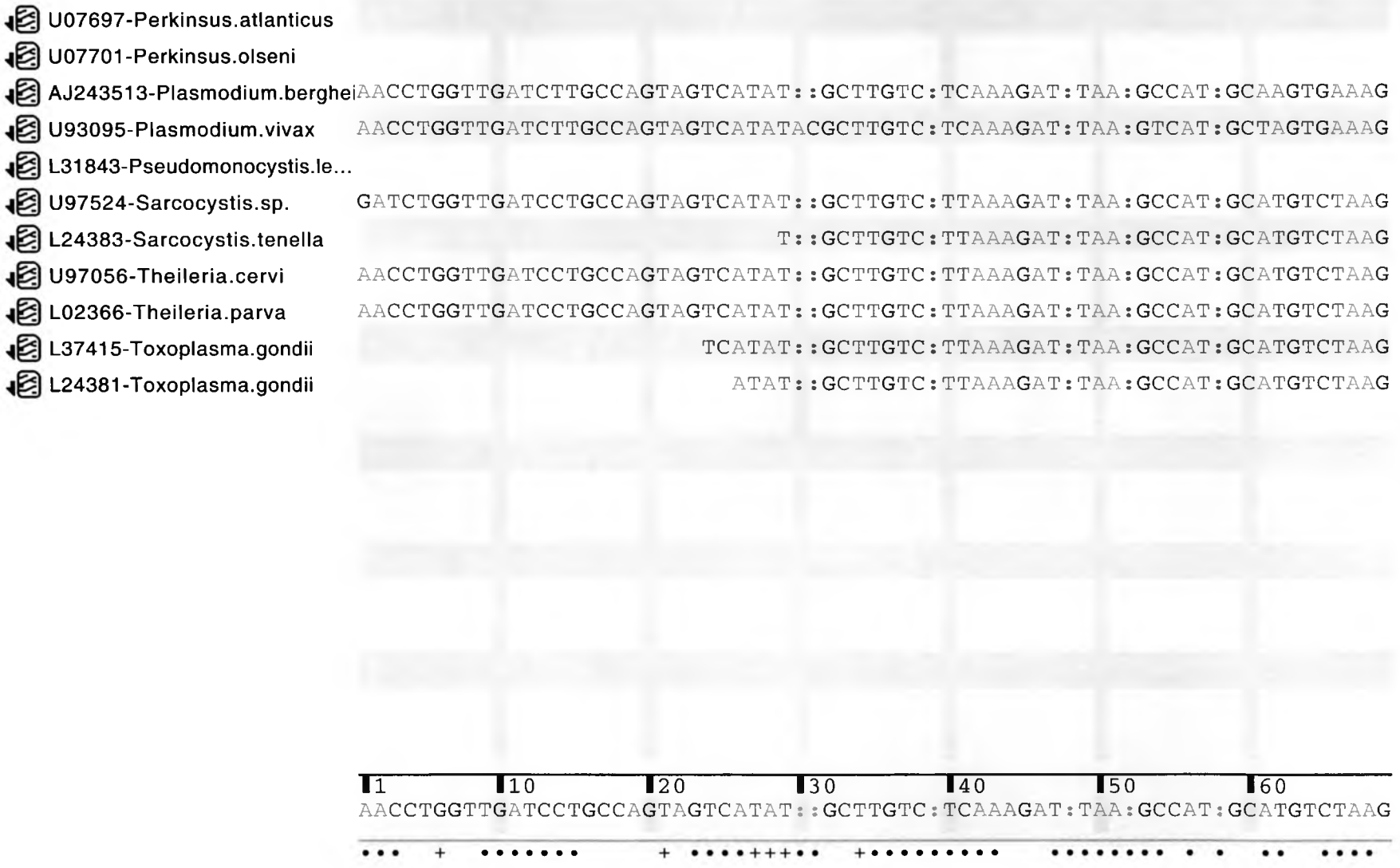
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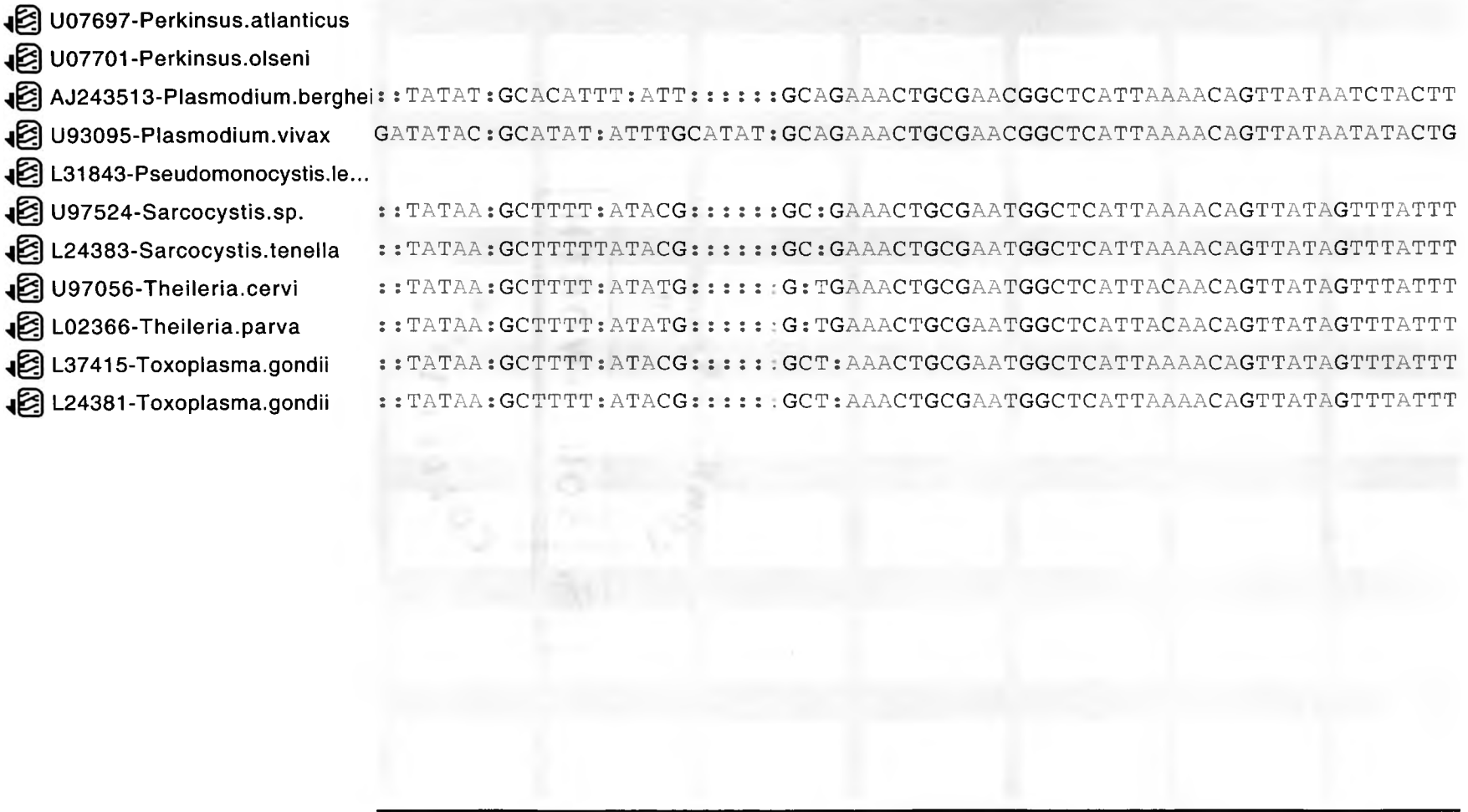
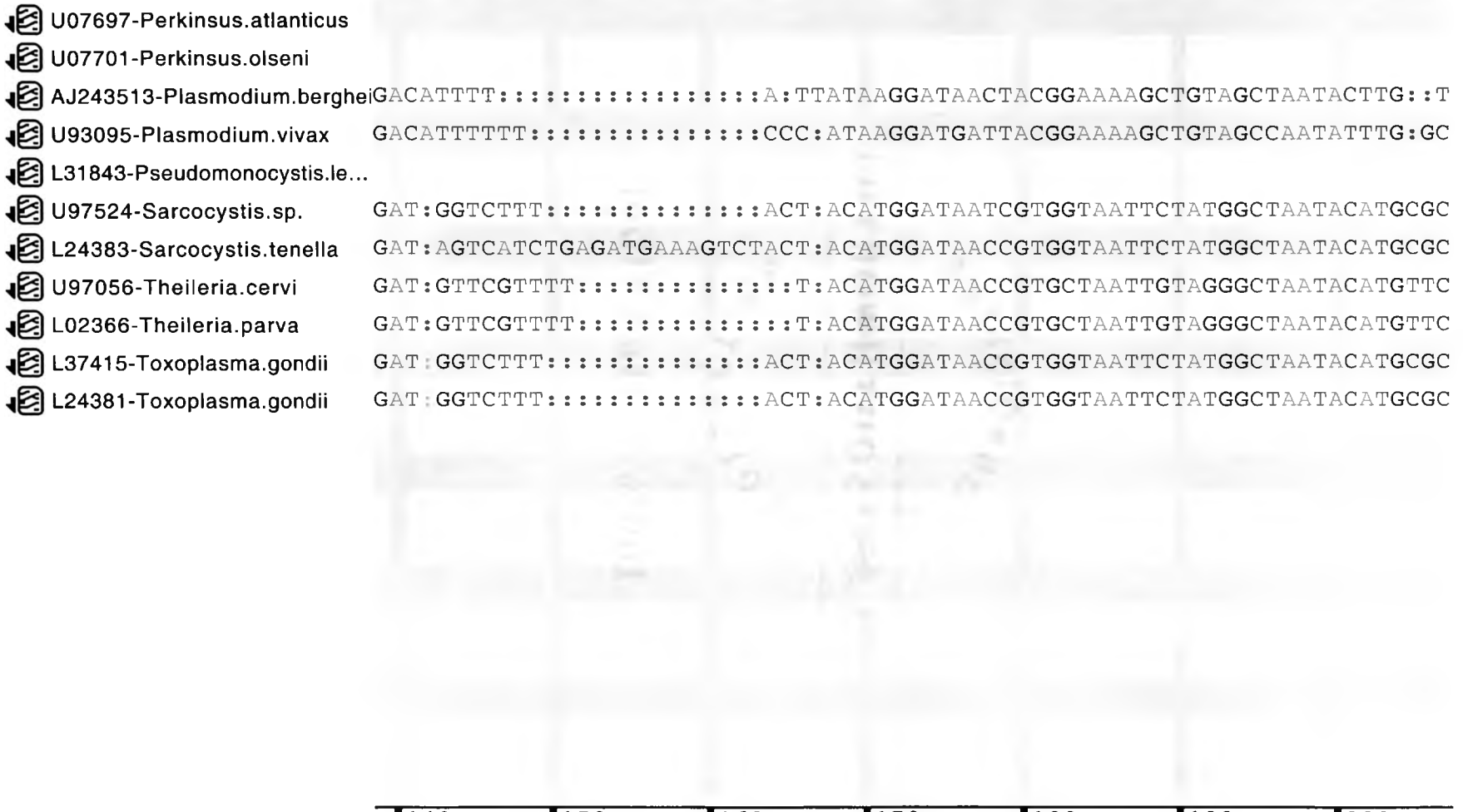
Appendix C (cont.)

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U97524-Sarcocystis.sp.
L24383-Sarcocystis.tenella
U97056-Theileria.cervi
L02366-Theileria.parva
L37415-Toxoplasma.gondii
L24381-Toxoplasma.gondii
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U97524-Sarcocystis.sp.
L24383-Sarcocystis.tenella
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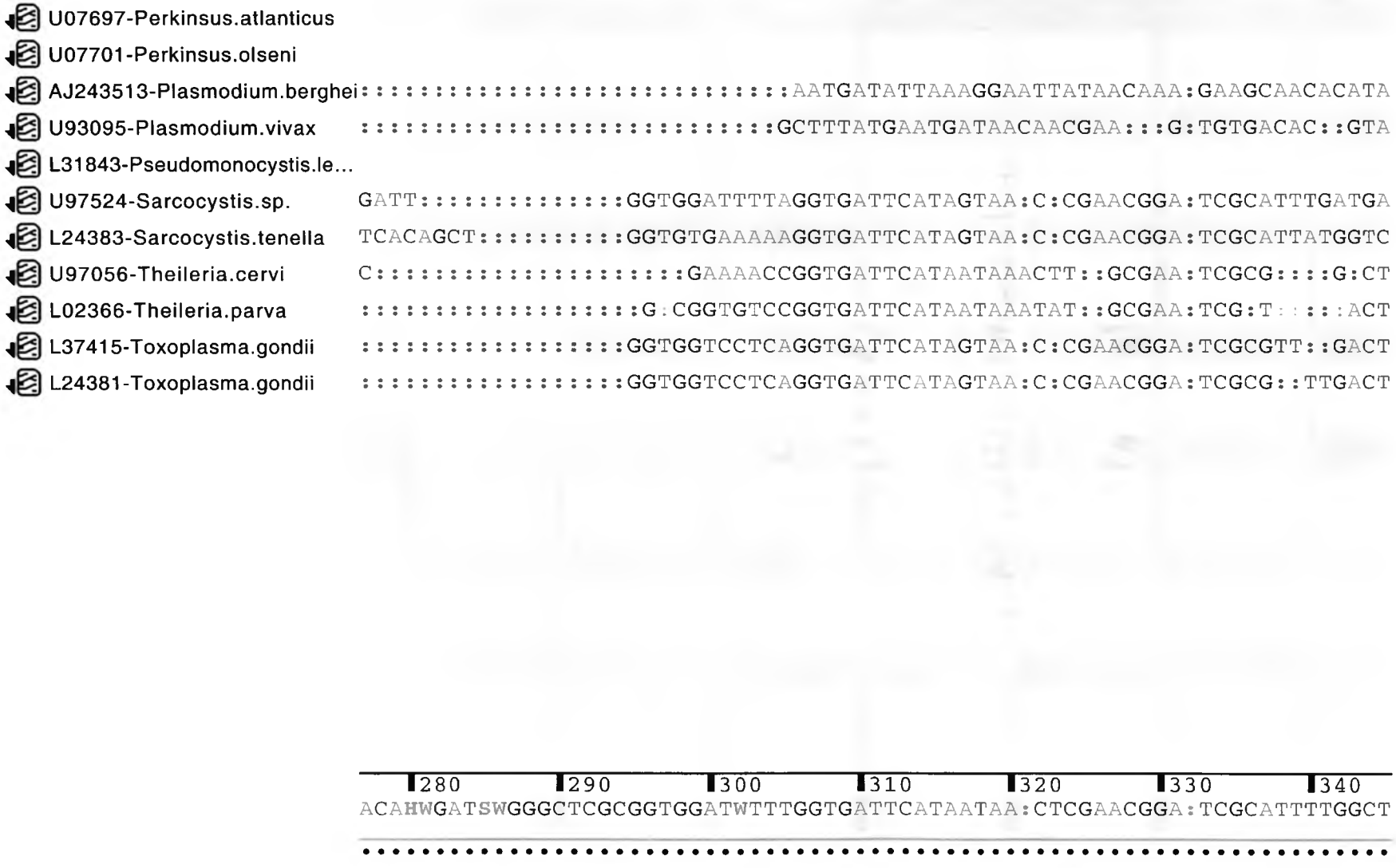
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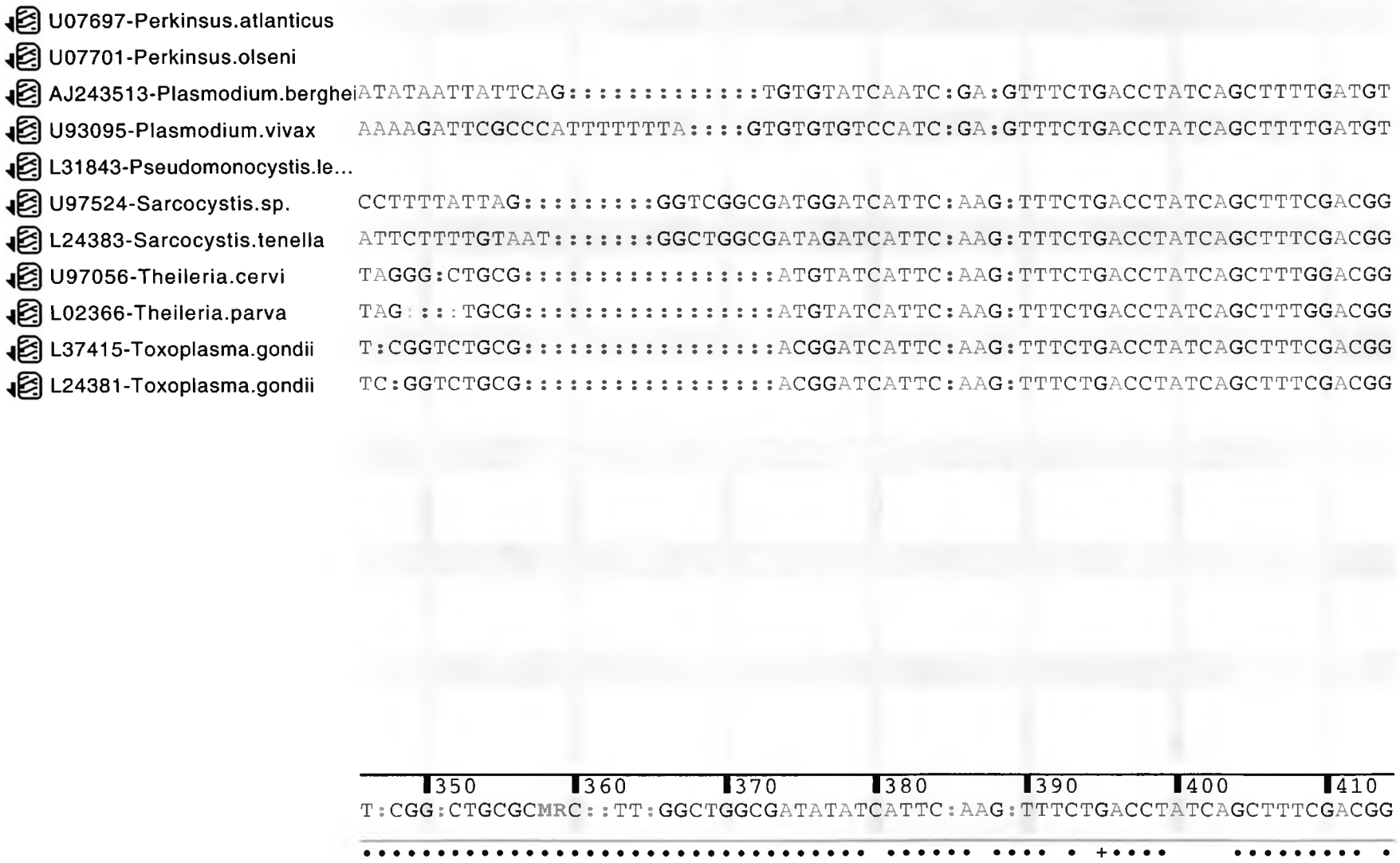
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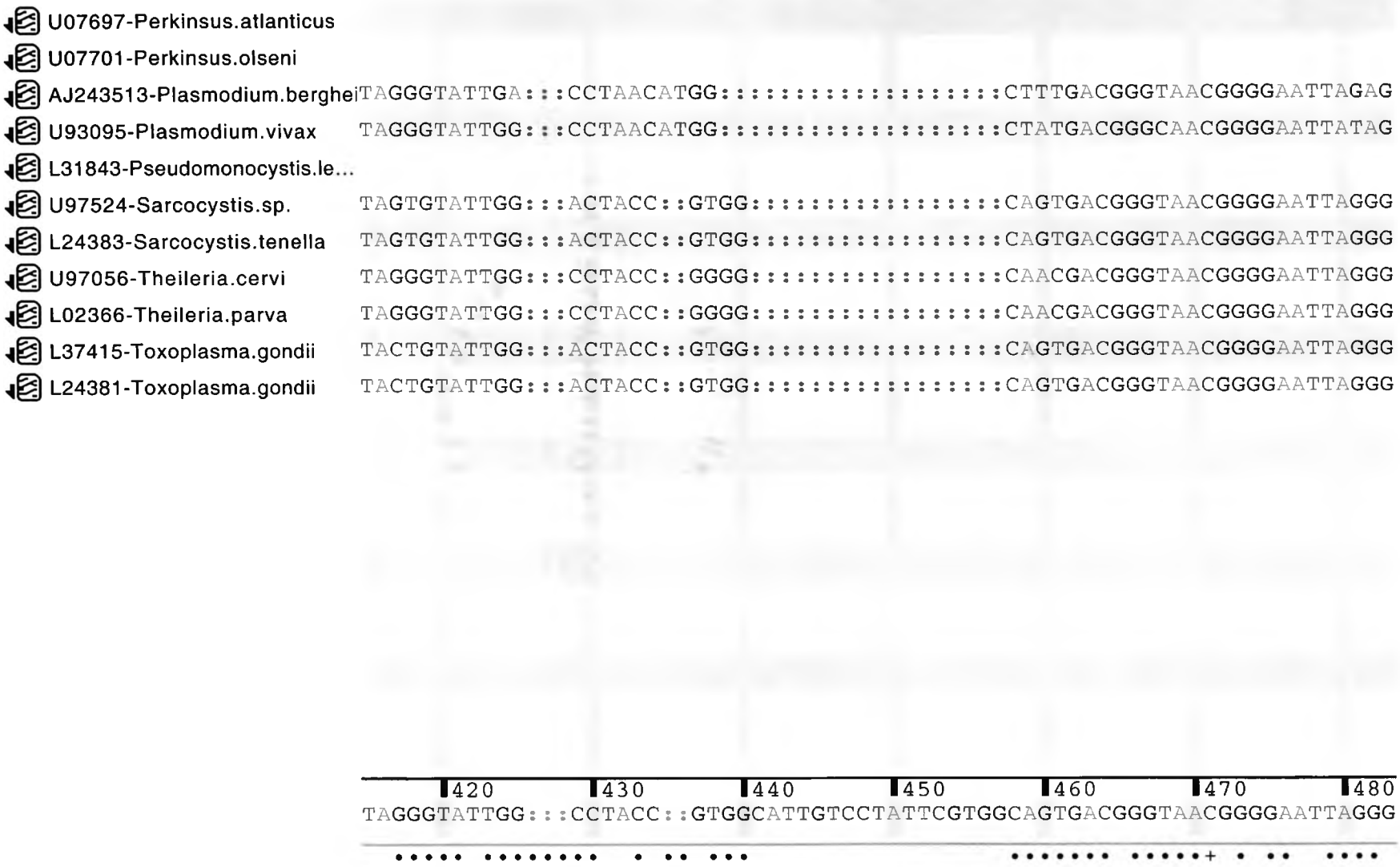
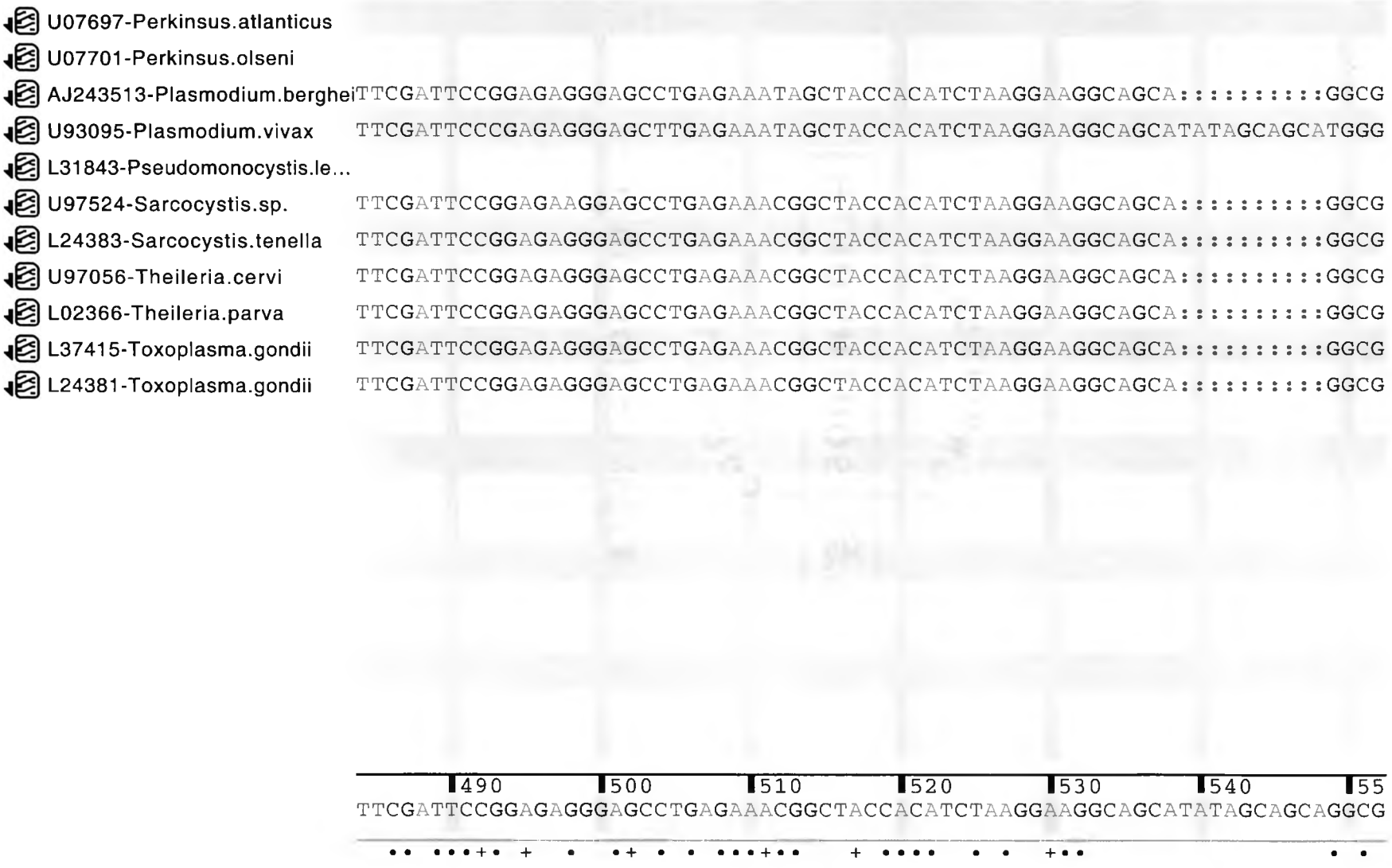
Appendix C (cont.)

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U97524-Sarcocystis.sp. TTCGATTCCGGAGAAGGAGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCA:::::::::GGCG
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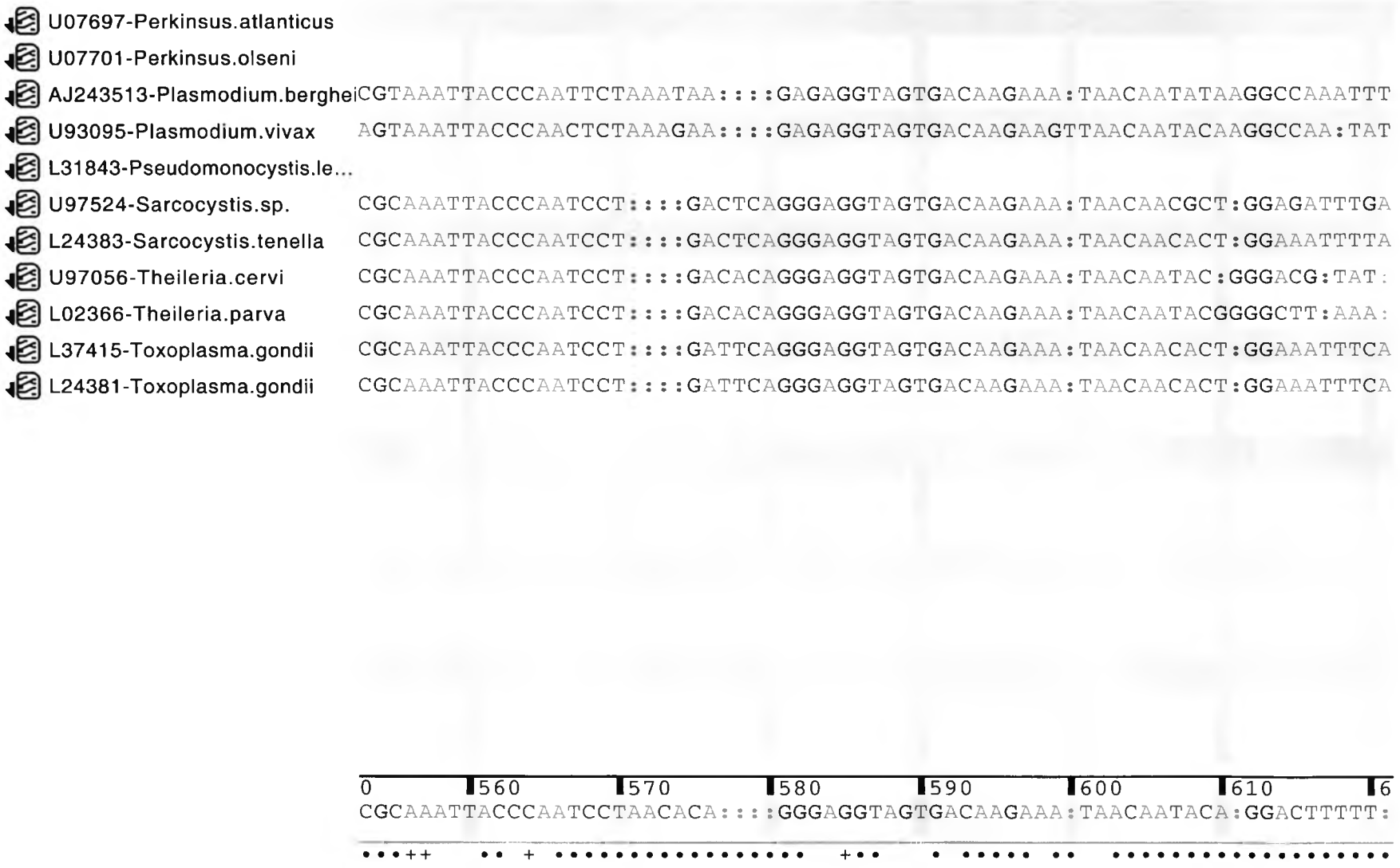
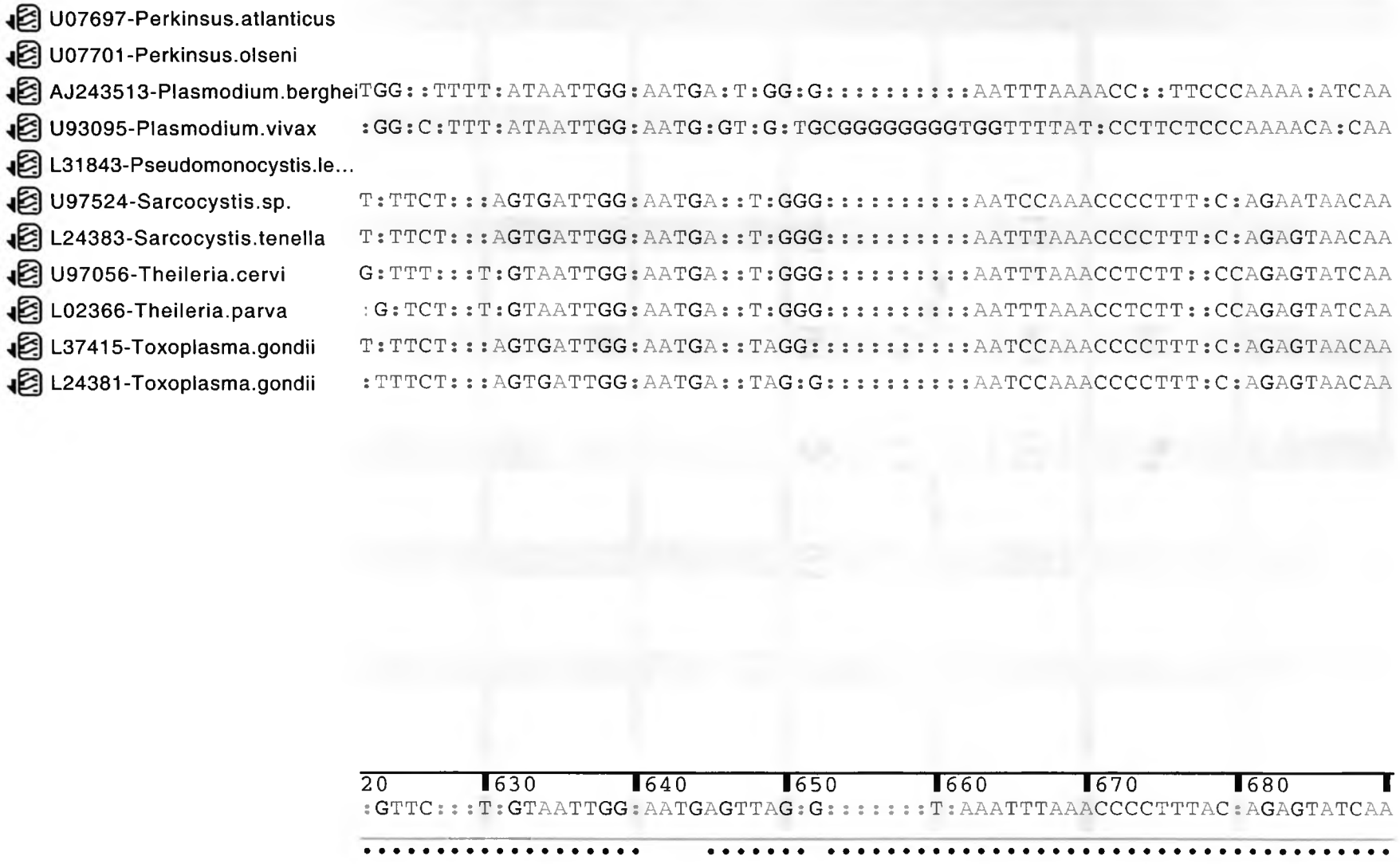
Appendix C (cont.)

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L31843-Pseudomonocystis.le...
U97524-Sarcocystis.sp.
L24383-Sarcocystis.tenella
U97056-Theileria.cervi
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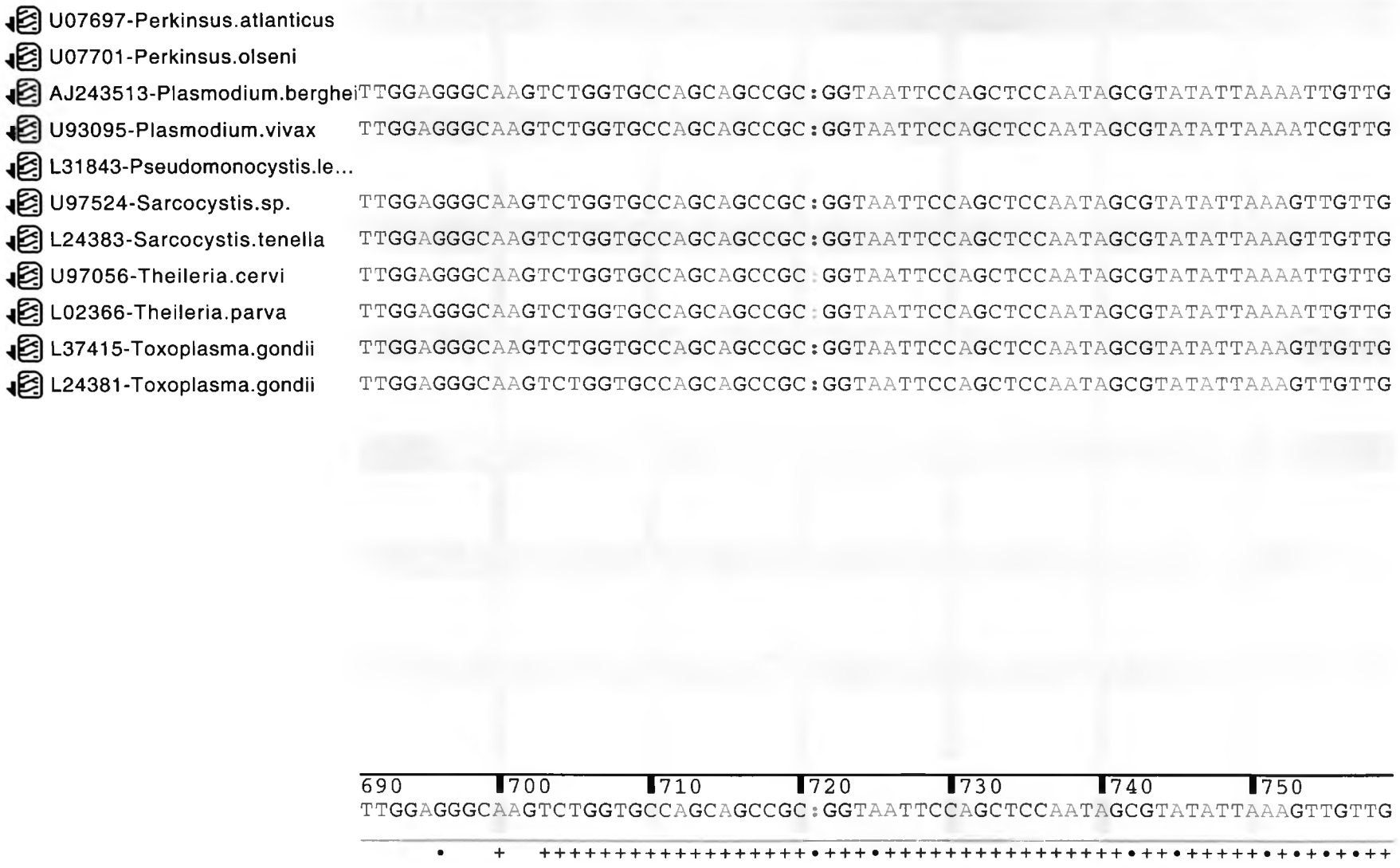
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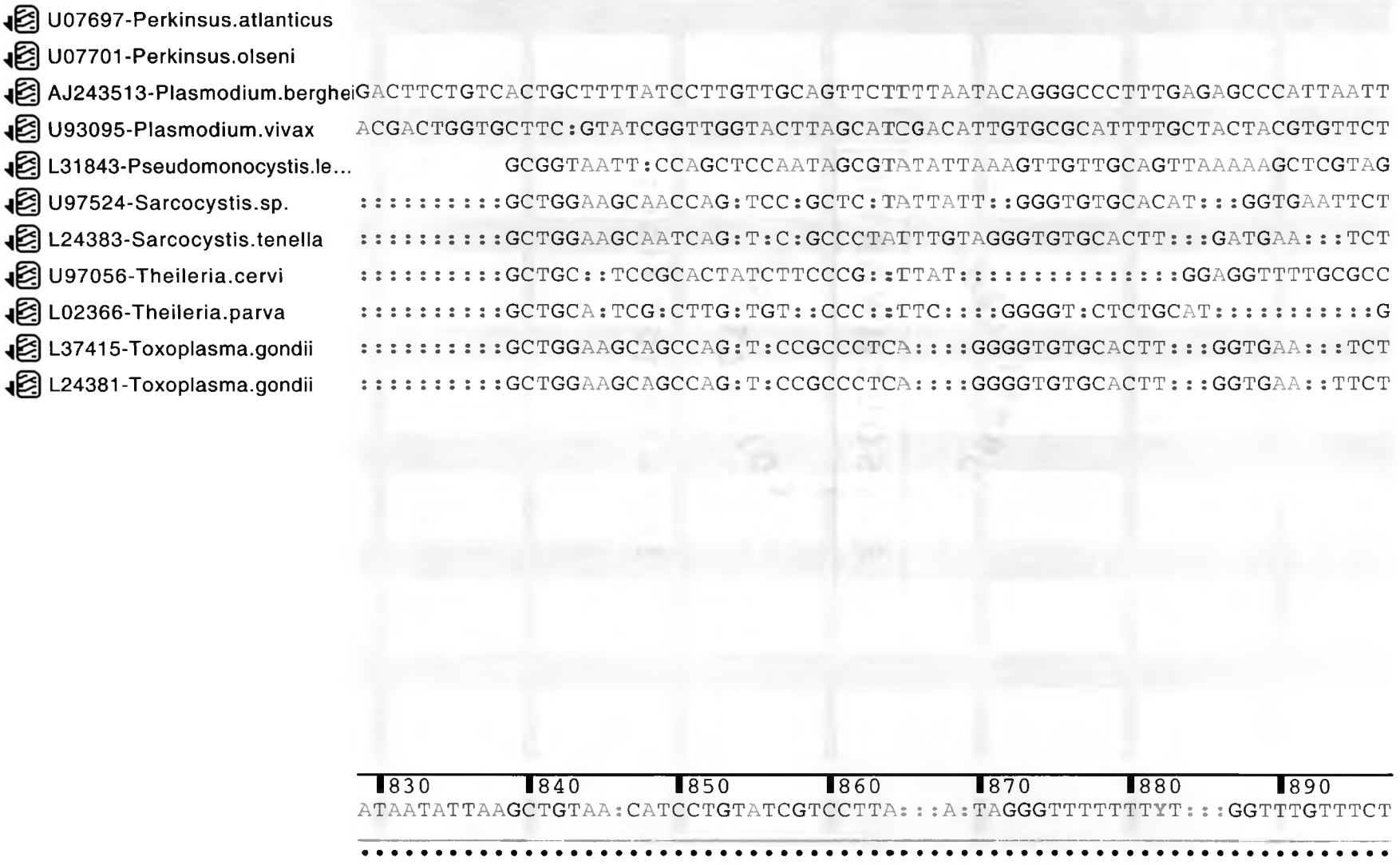
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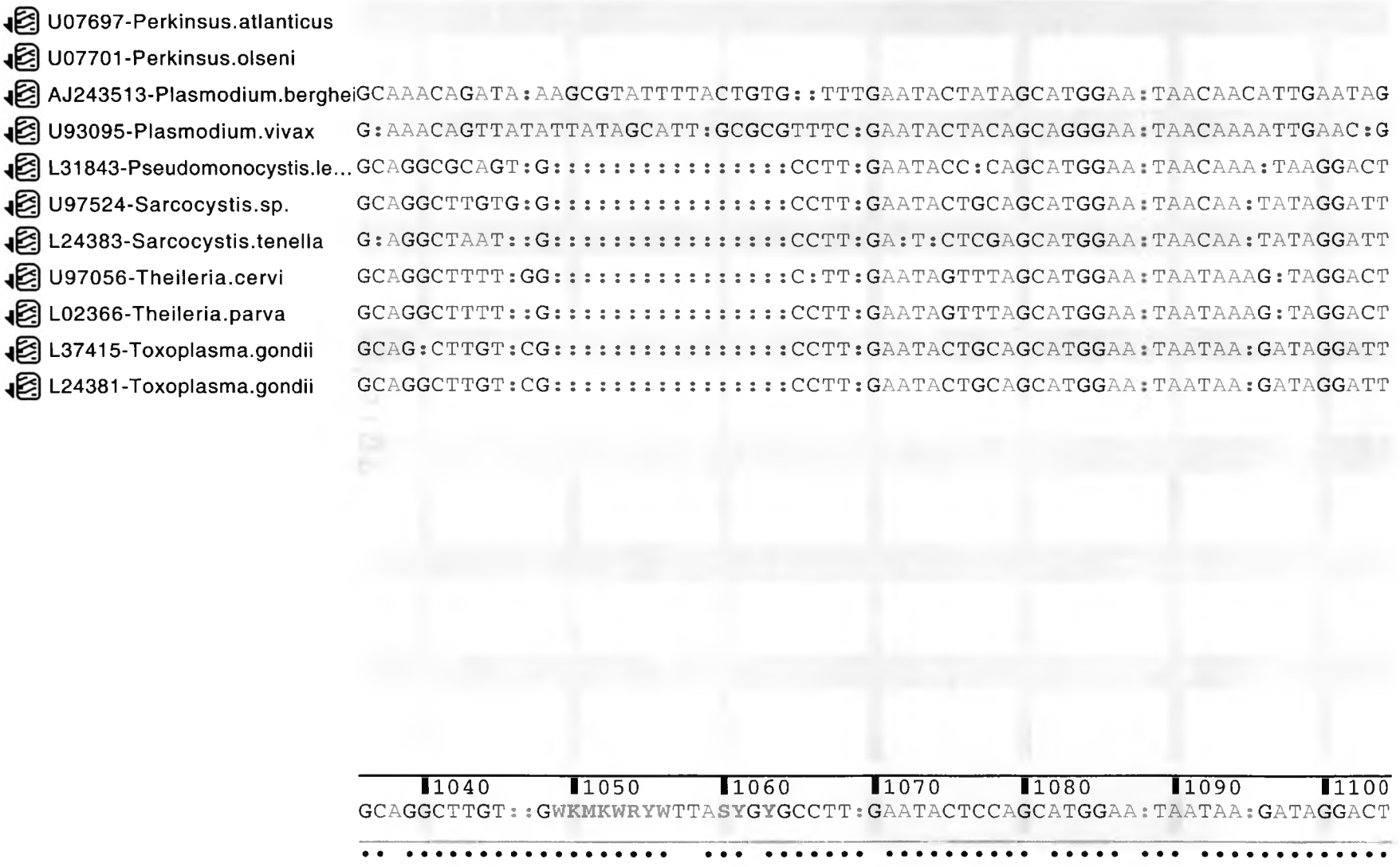
Appendix C (cont.)

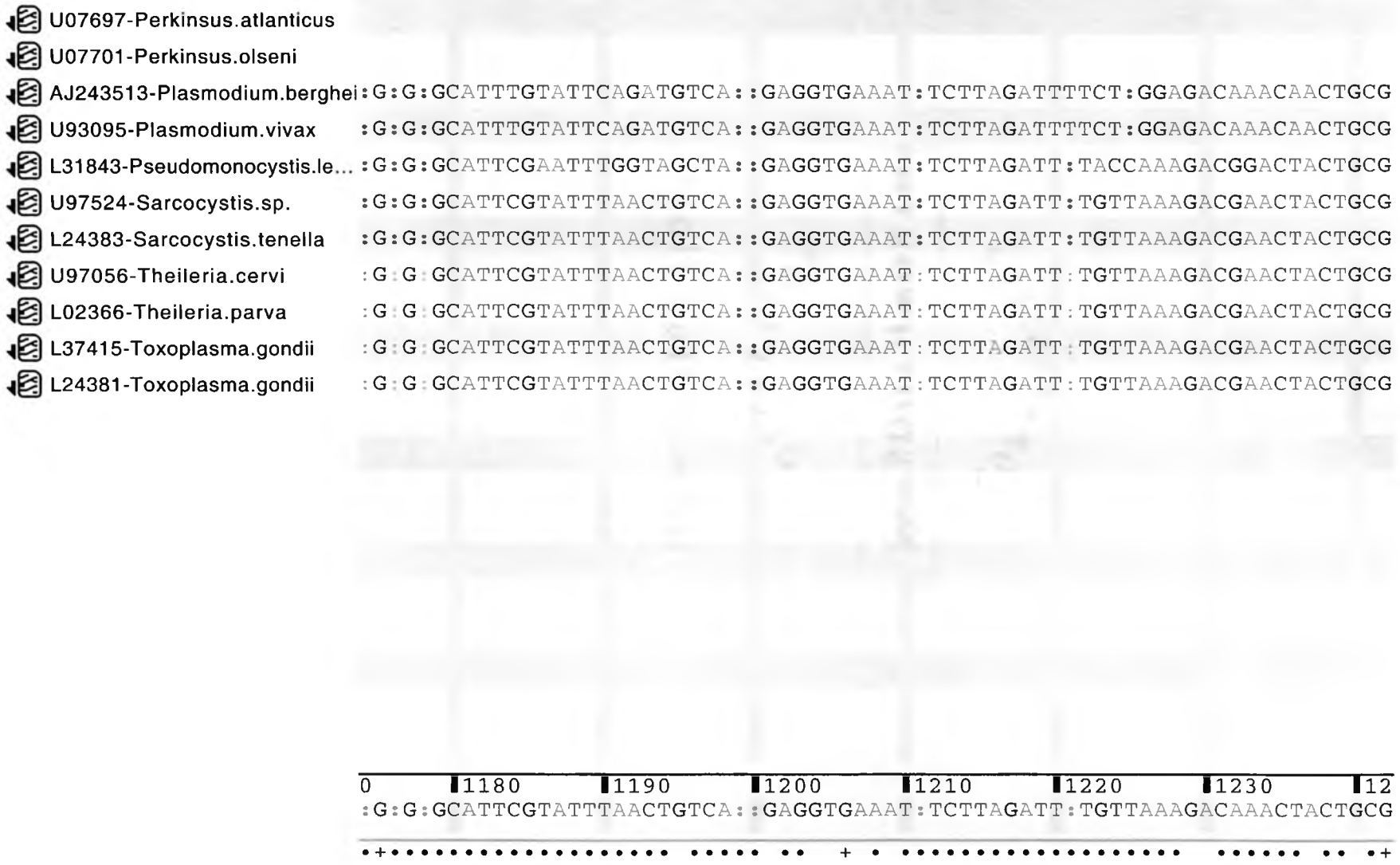
Appendix C (cont.)

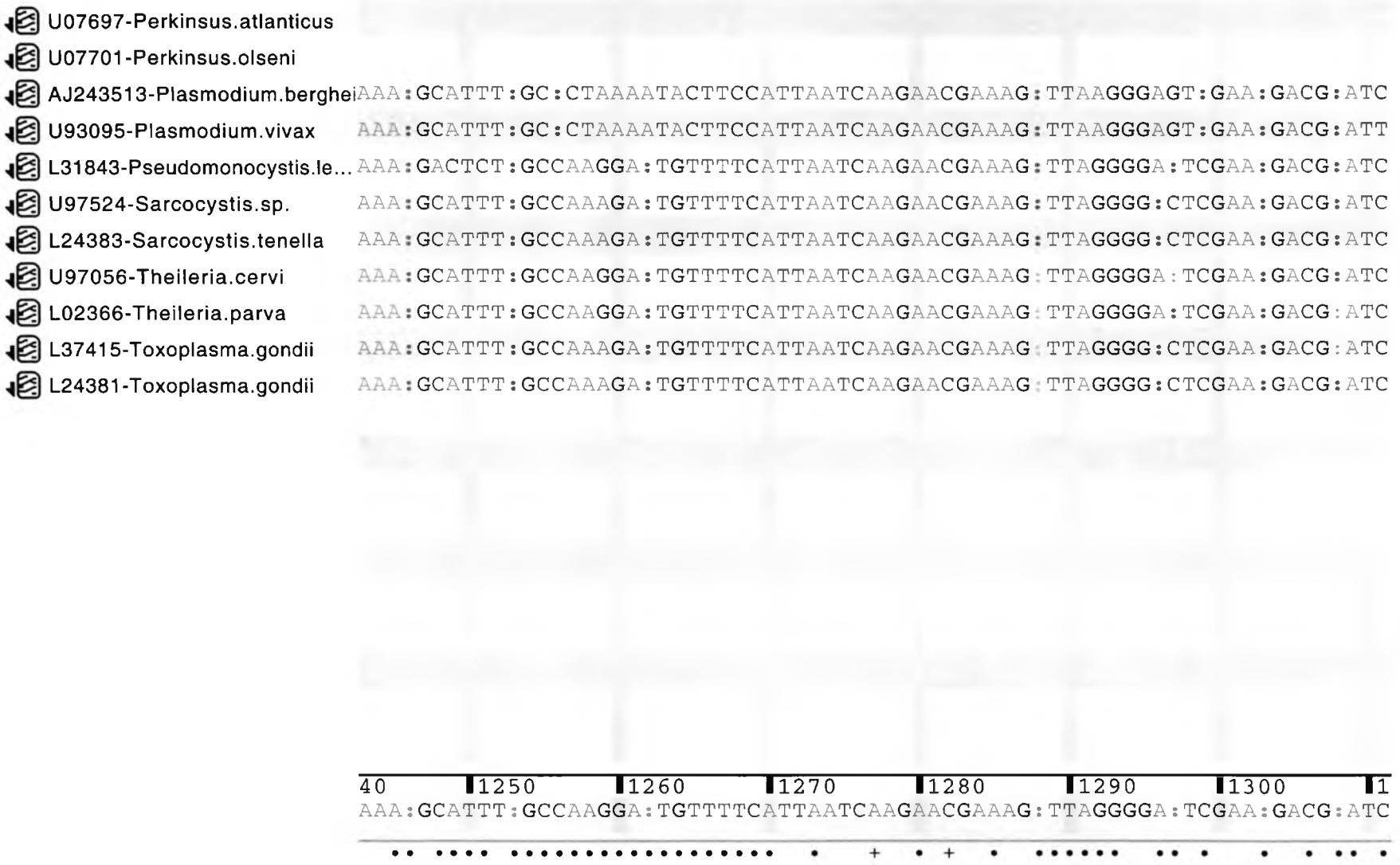
Appendix C (cont.)



Appendix C (cont.)

Appendix C (cont.)

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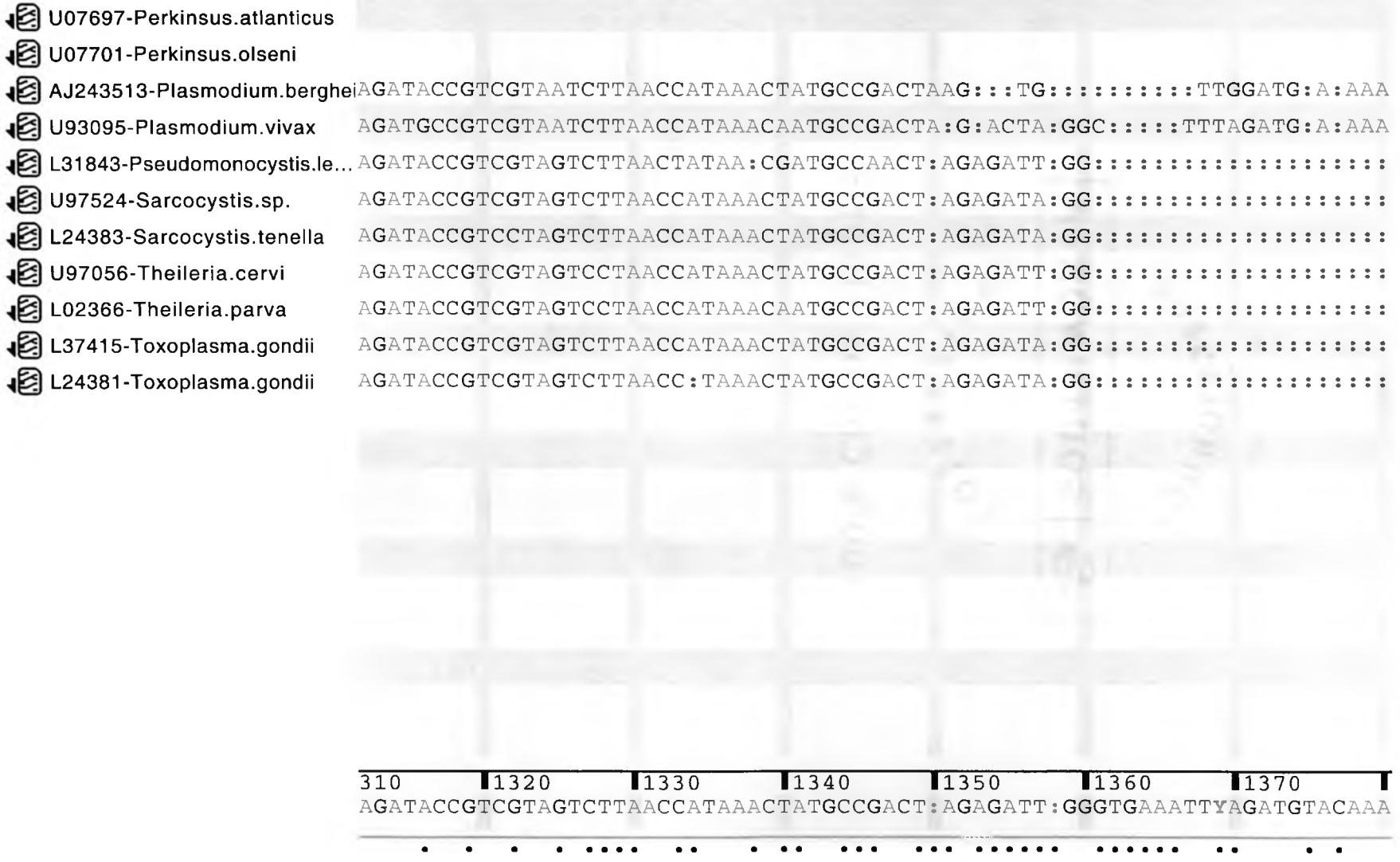
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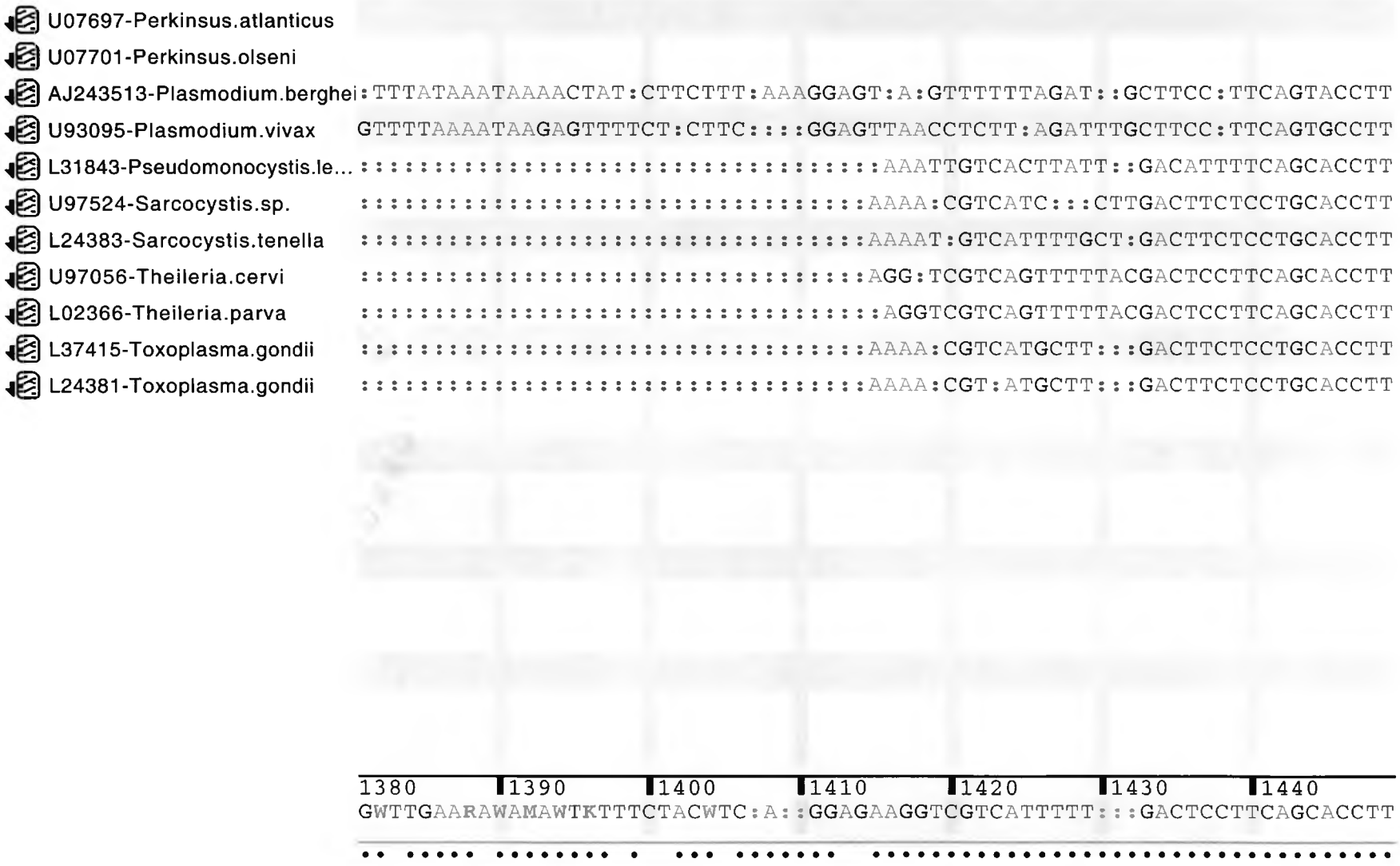
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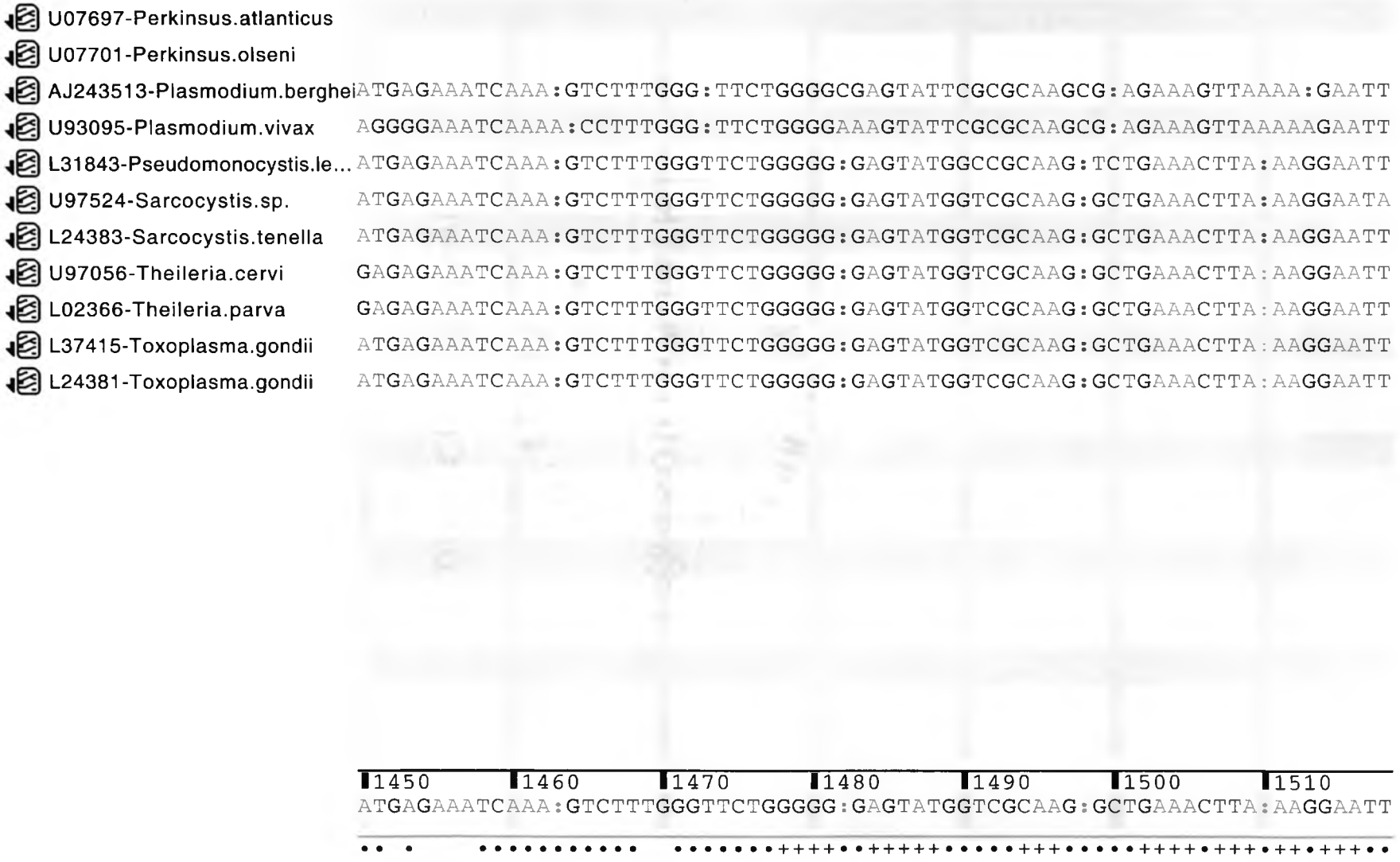
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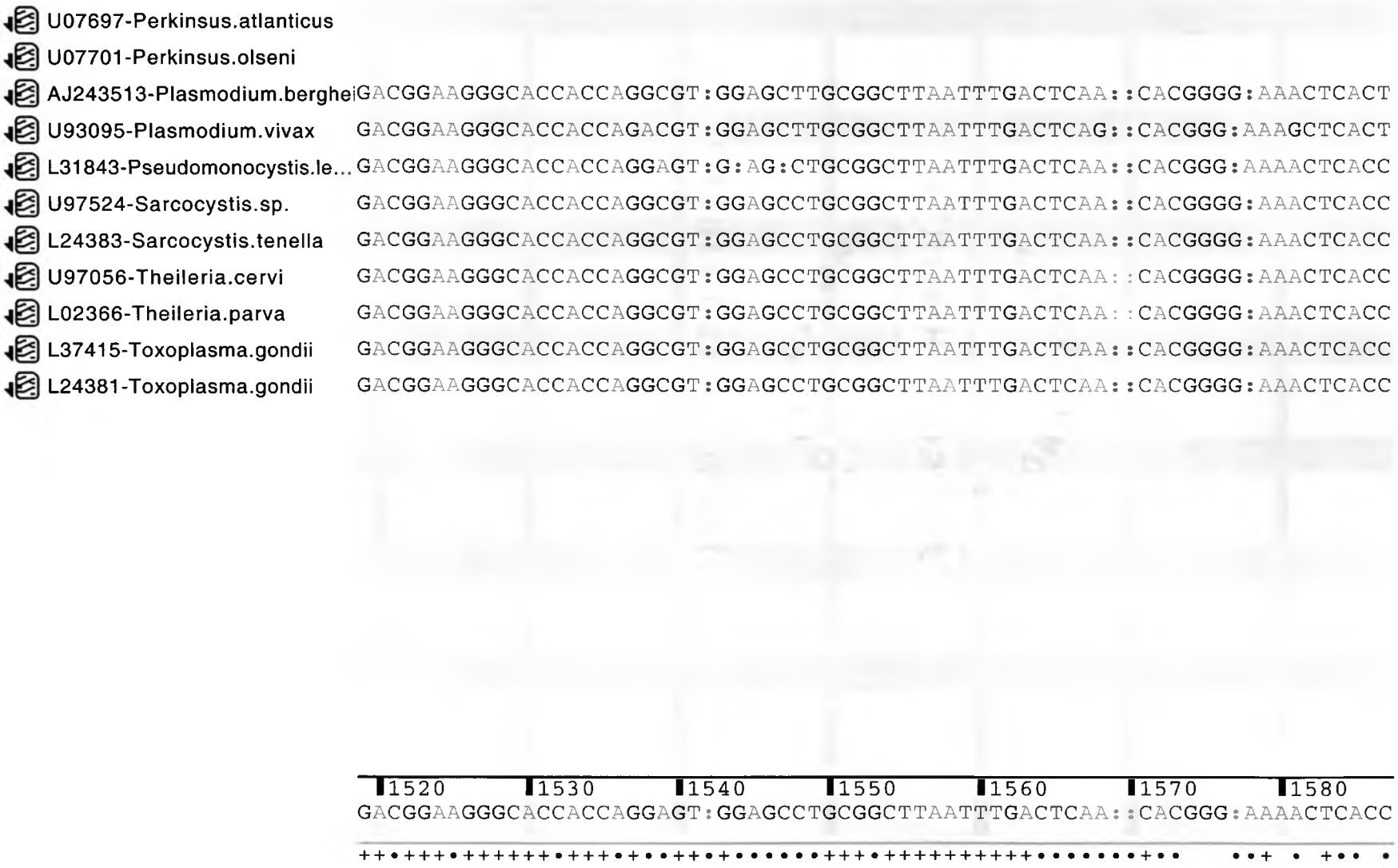
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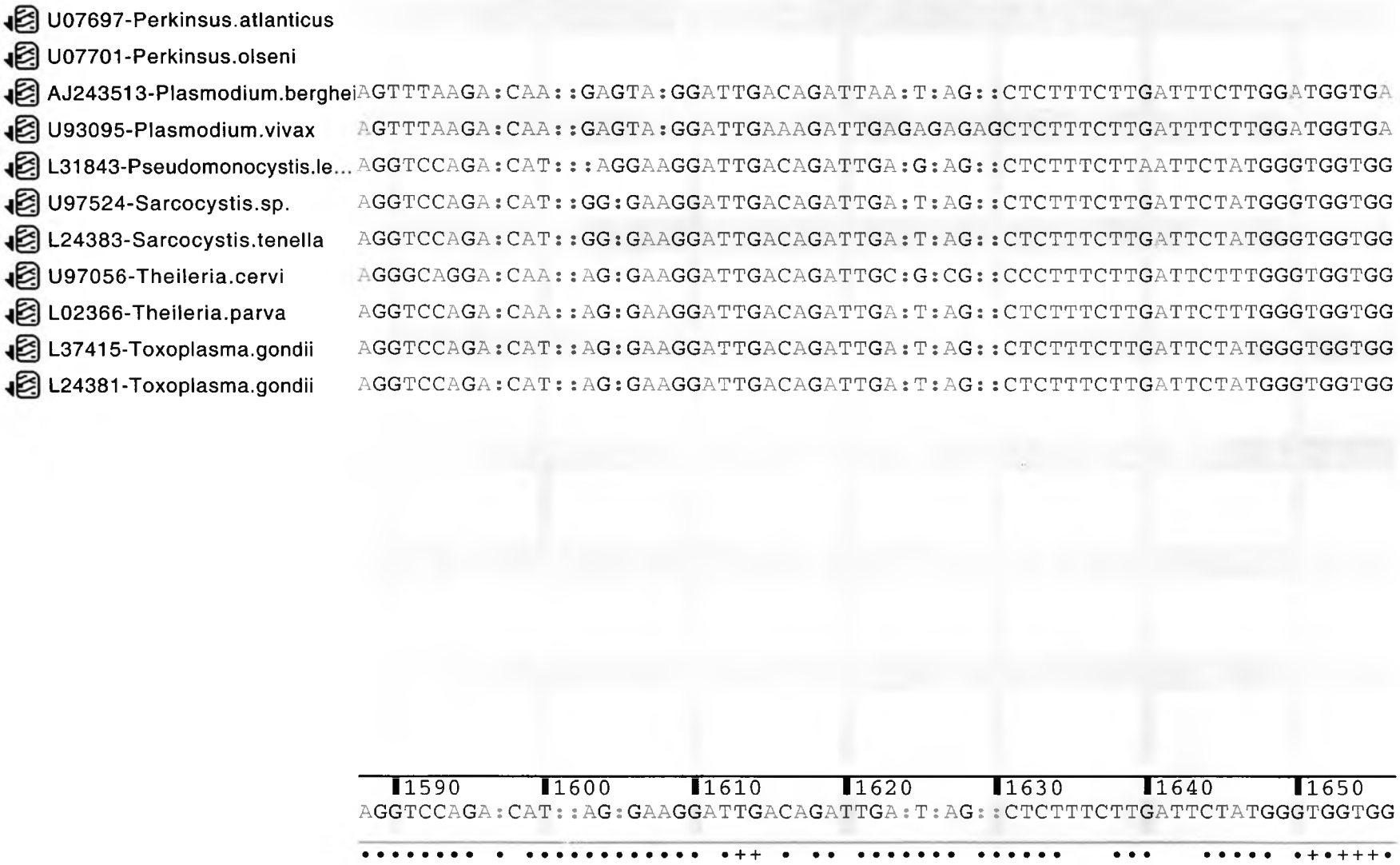
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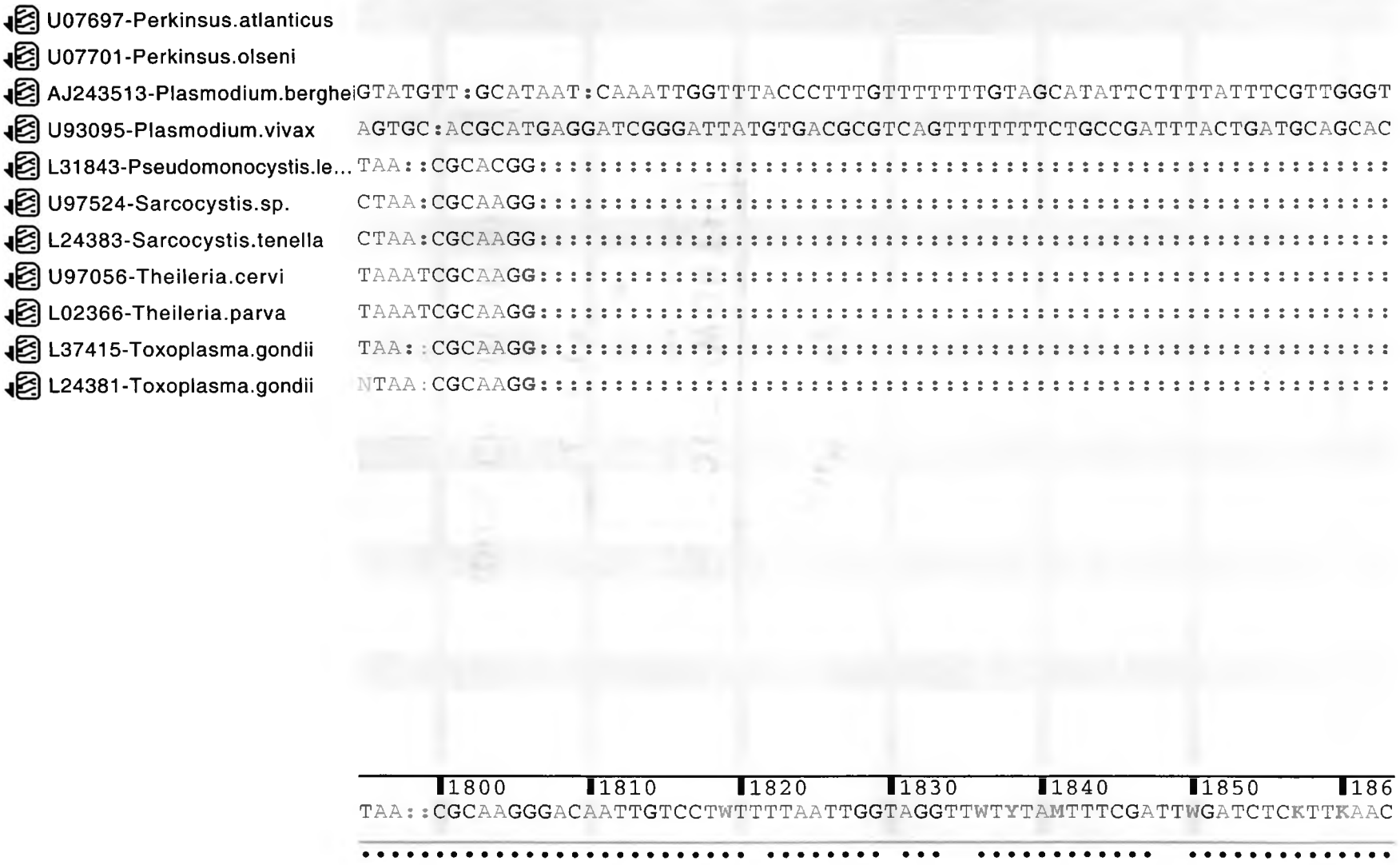
Appendix C (cont.)

Appendix C (cont.)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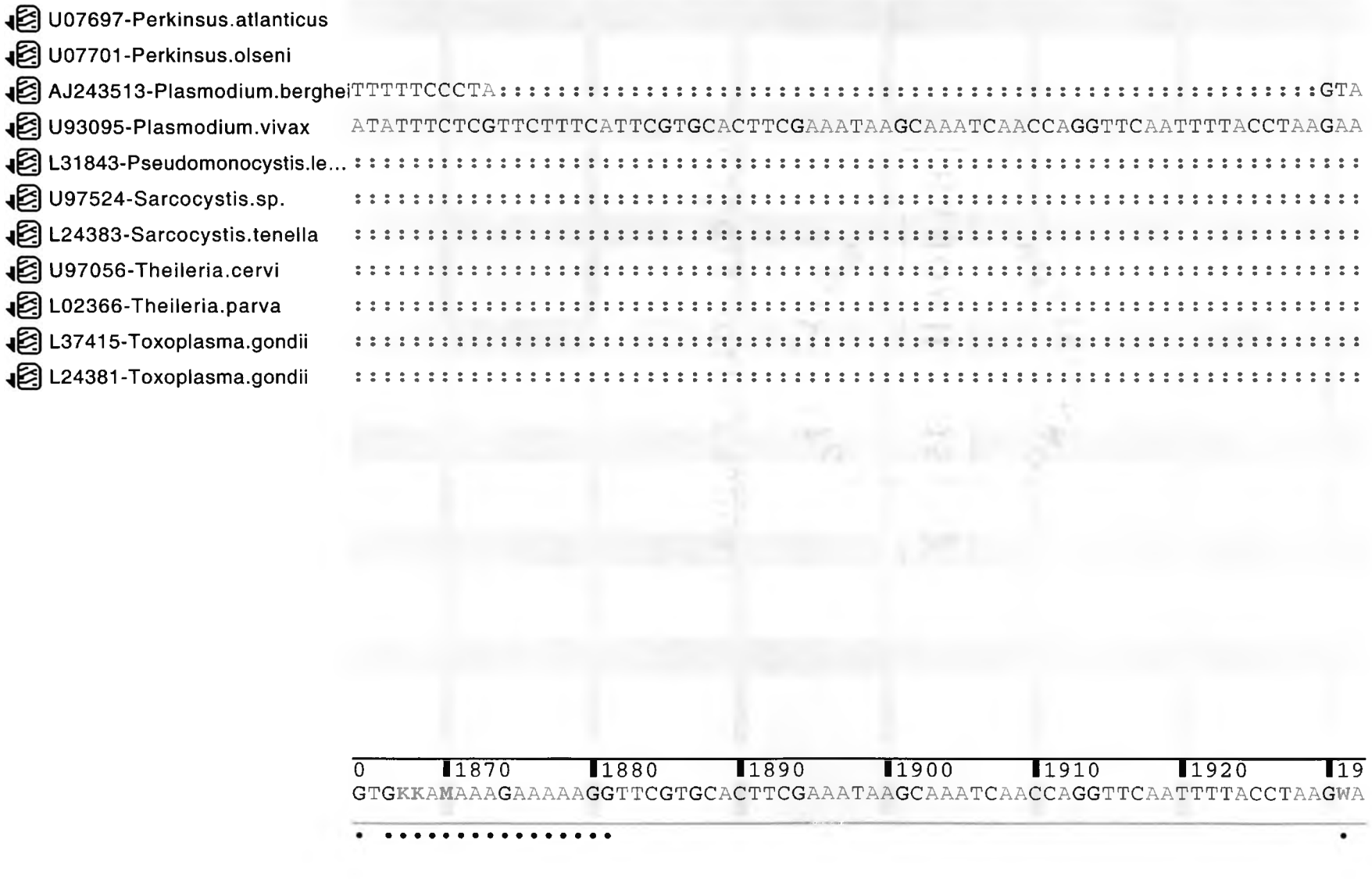
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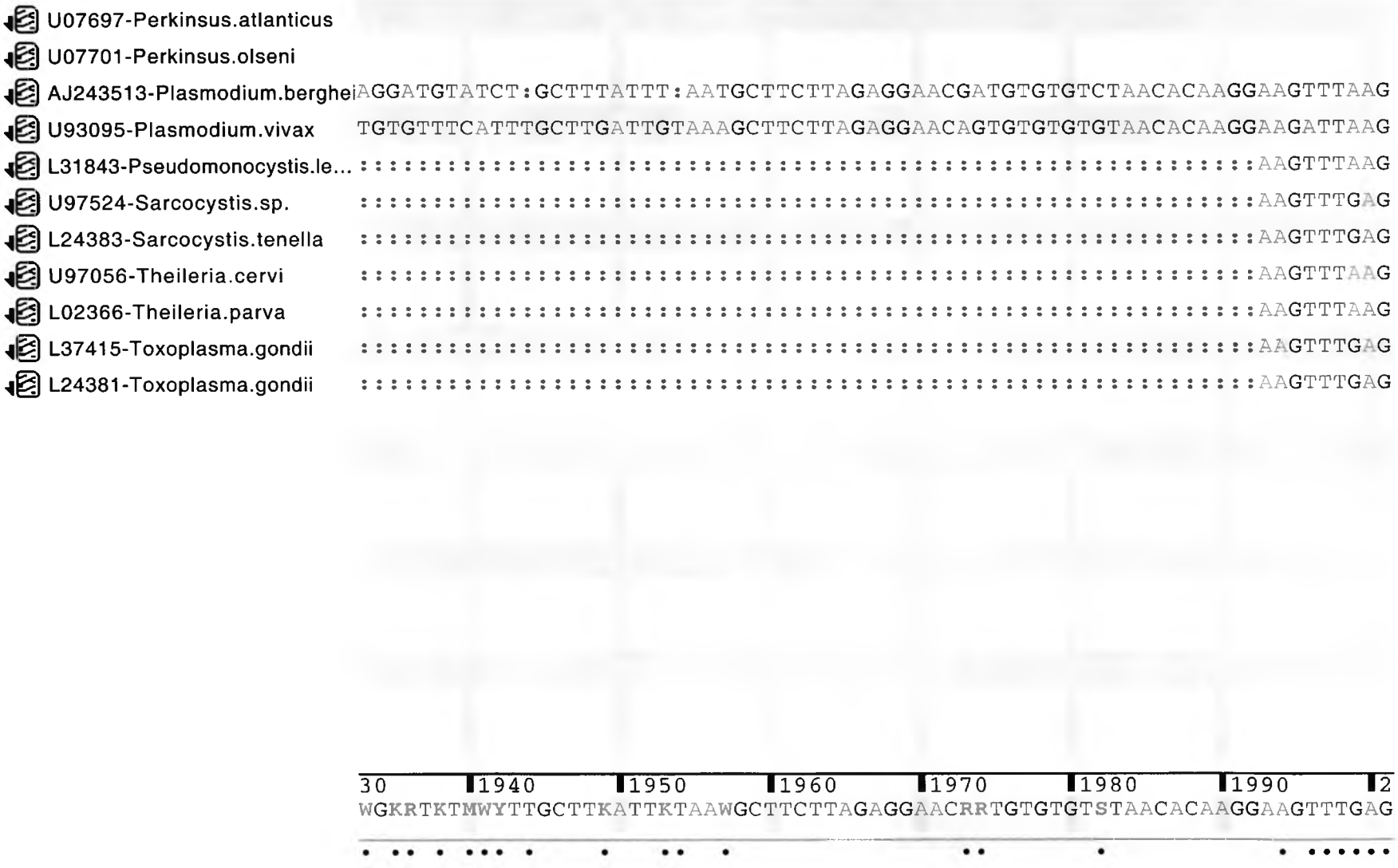
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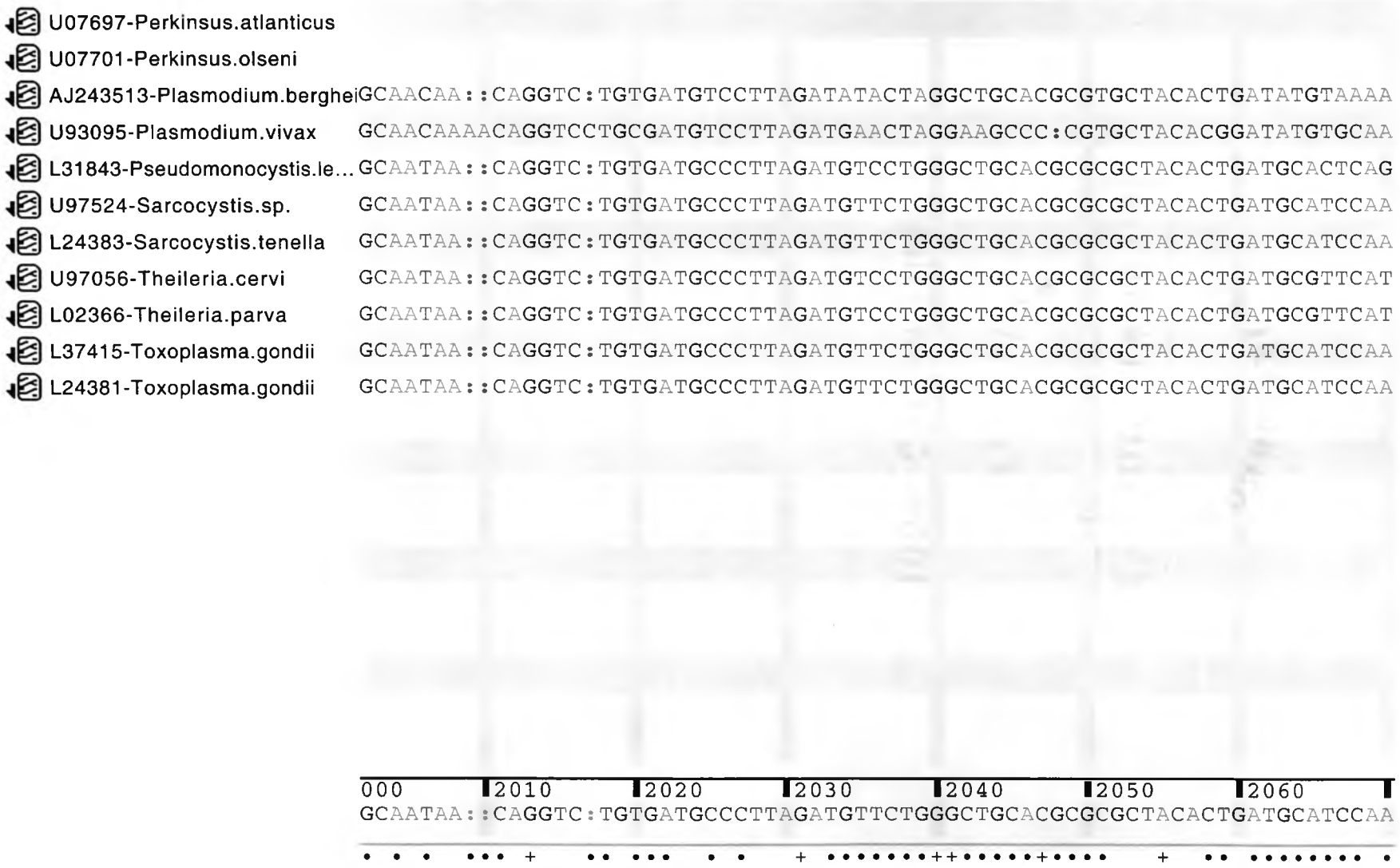
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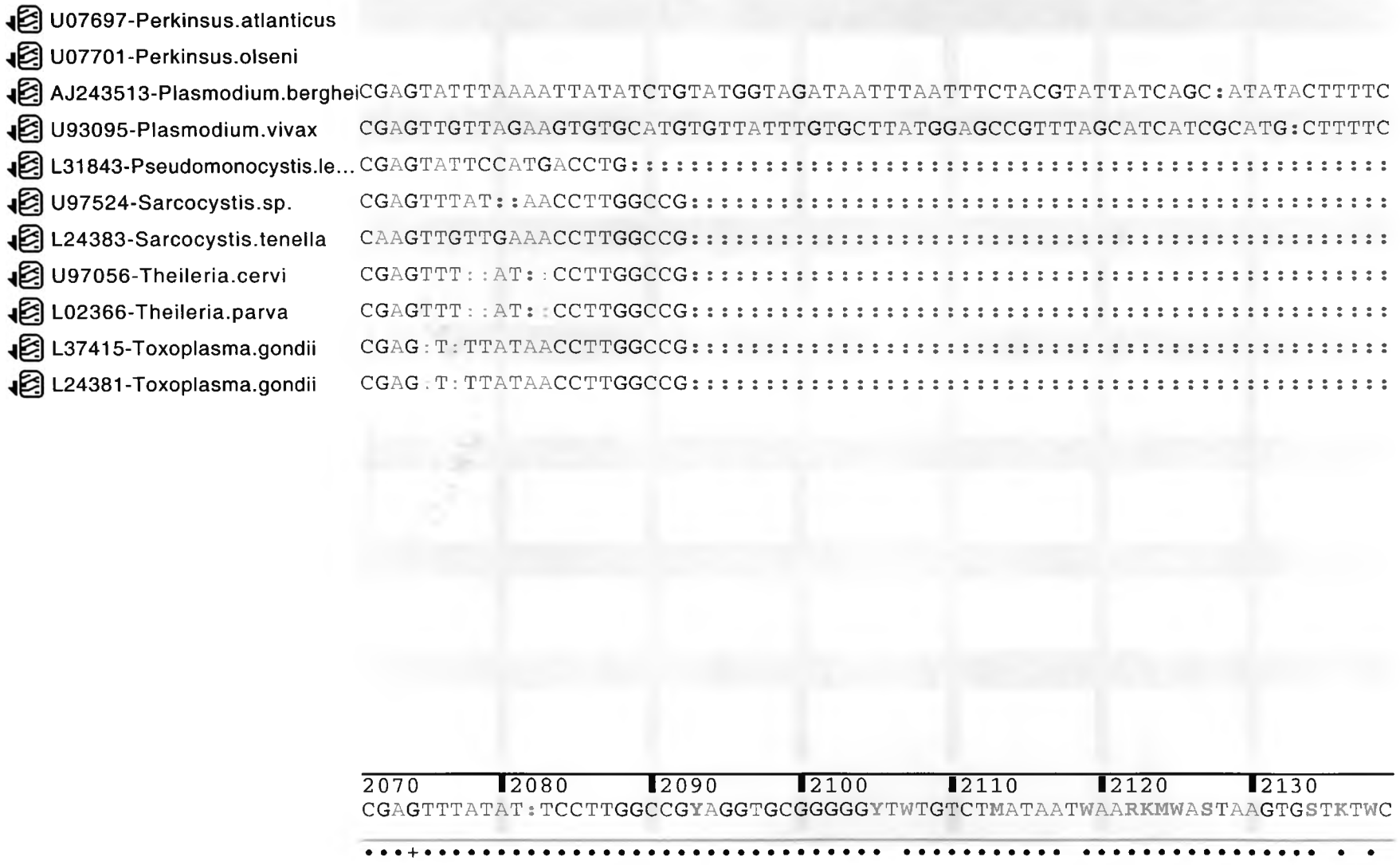
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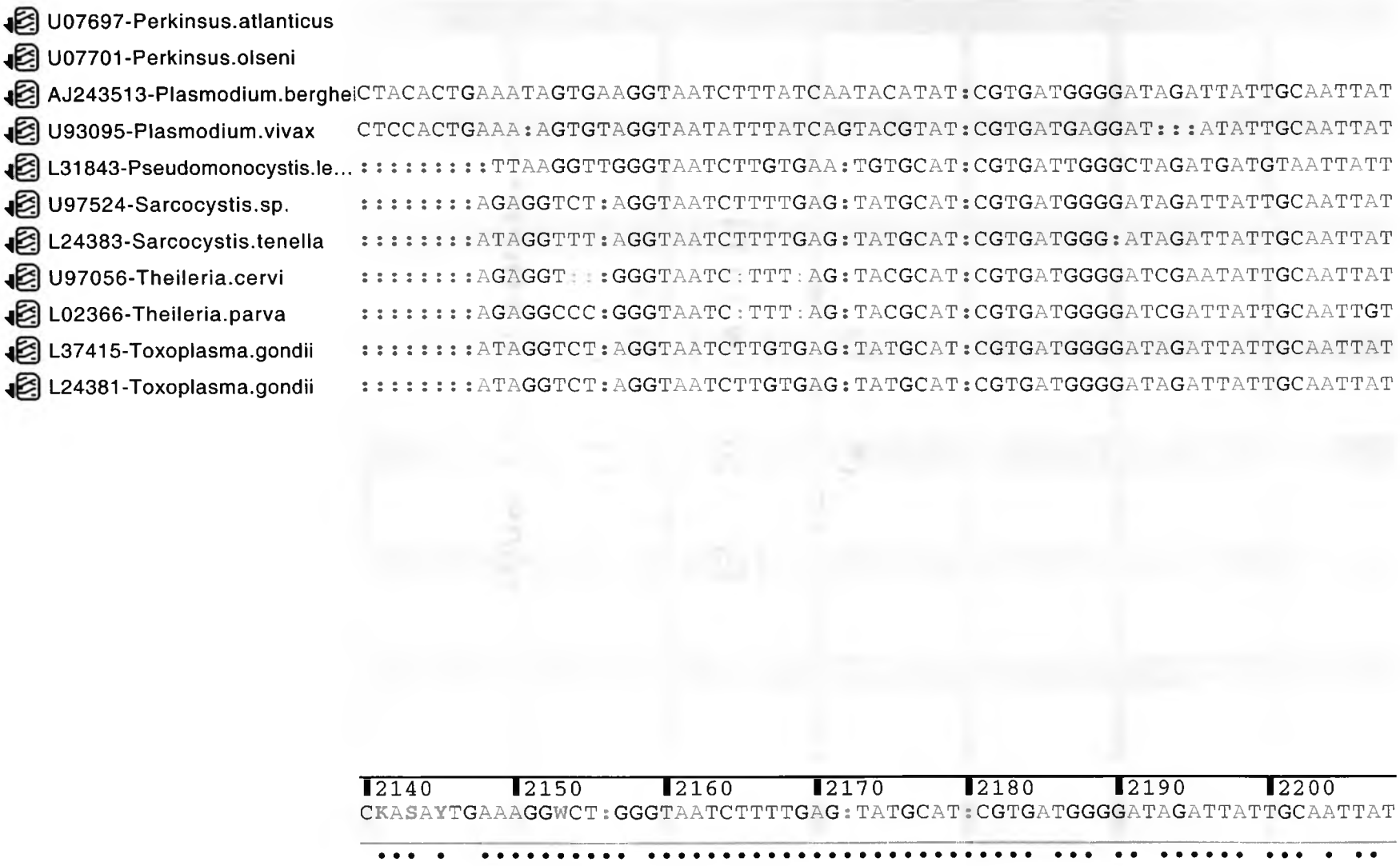
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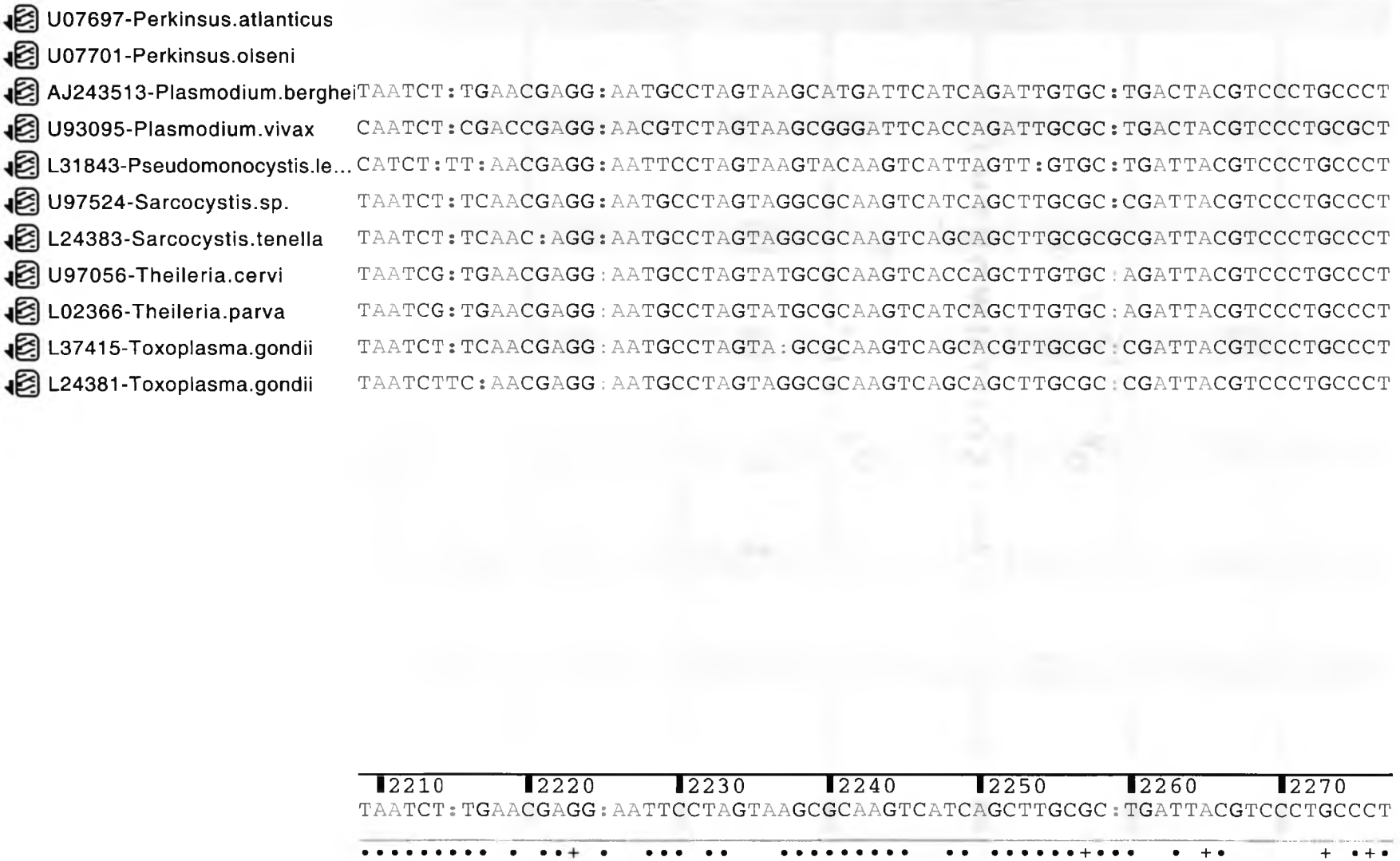
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U07701-Perkinsus.olseni
AJ243513-Plasmodium.berghei TAATCT:TGAACGAGG:AATGCCTAGTAAGCATGATTCATCAGATTGTGC:TGACTACGTCCCTGCCCT
U93095-Plasmodium.vivax CAATCT:CGACCGAGG:AACGTCTAGTAAGCGGGATTCACCAGATTGCGC:TGACTACGTCCCTGCGCT
L31843-Pseudomonocystis.le... CATCT:TT:AACGAGG:AATTCCTAGTAAGTACAAGTCATTAGTT:GTGC:TGATTACGTCCCTGCCCT
U97524-Sarcocystis.sp. TAATCT:TCAACGAGG:AATGCCTAGTAGGCGCAAGTCATCAGCTTGCGC:CGATTACGTCCCTGCCCT
L24383-Sarcocystis.tenella TAATCT:TCAAC:AGG:AATGCCTAGTAGGCGCAAGTCAGCAGCTTGCGCGCGATTACGTCCCTGCCCT
U97056-Theileria.cervi TAATCG:TGAACGAGG:AATGCCTAGTATGCGCAAGTCACCAGCTTGTGC:AGATTACGTCCCTGCCCT
L02366-Theileria.parva TAATCG:TGAACGAGG:AATGCCTAGTATGCGCAAGTCATCAGCTTGTGC:AGATTACGTCCCTGCCCT
L37415-Toxoplasma.gondii TAATCT:TCAACGAGG:AATGCCTAGTA:GCGCAAGTCAGCACGTTGCGC:CGATTACGTCCCTGCCCT
L24381-Toxoplasma.gondii TAATCTTC:AACGAGG:AATGCCTAGTAGGCGCAAGTCAGCAGCTTGCGC:CGATTACGTCCCTGCCCT
2210
2220
2230
2240
2250
2260
2270
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Appendix C (cont.)


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U97056-Theileria.cervi GT:TC::CCGTCAGGGAACGTCTAGGGAAGTTTTGTGAACCTTATCA:CTTA:AAGGAAGGAGAA:GTC
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L24381-Toxoplasma.gondii G:GC:GC:GT:TCGTGCCCGAAATGNGAAGTTTTGTGAACCTTAACA:CTTA:GAGGAAGGAGAA
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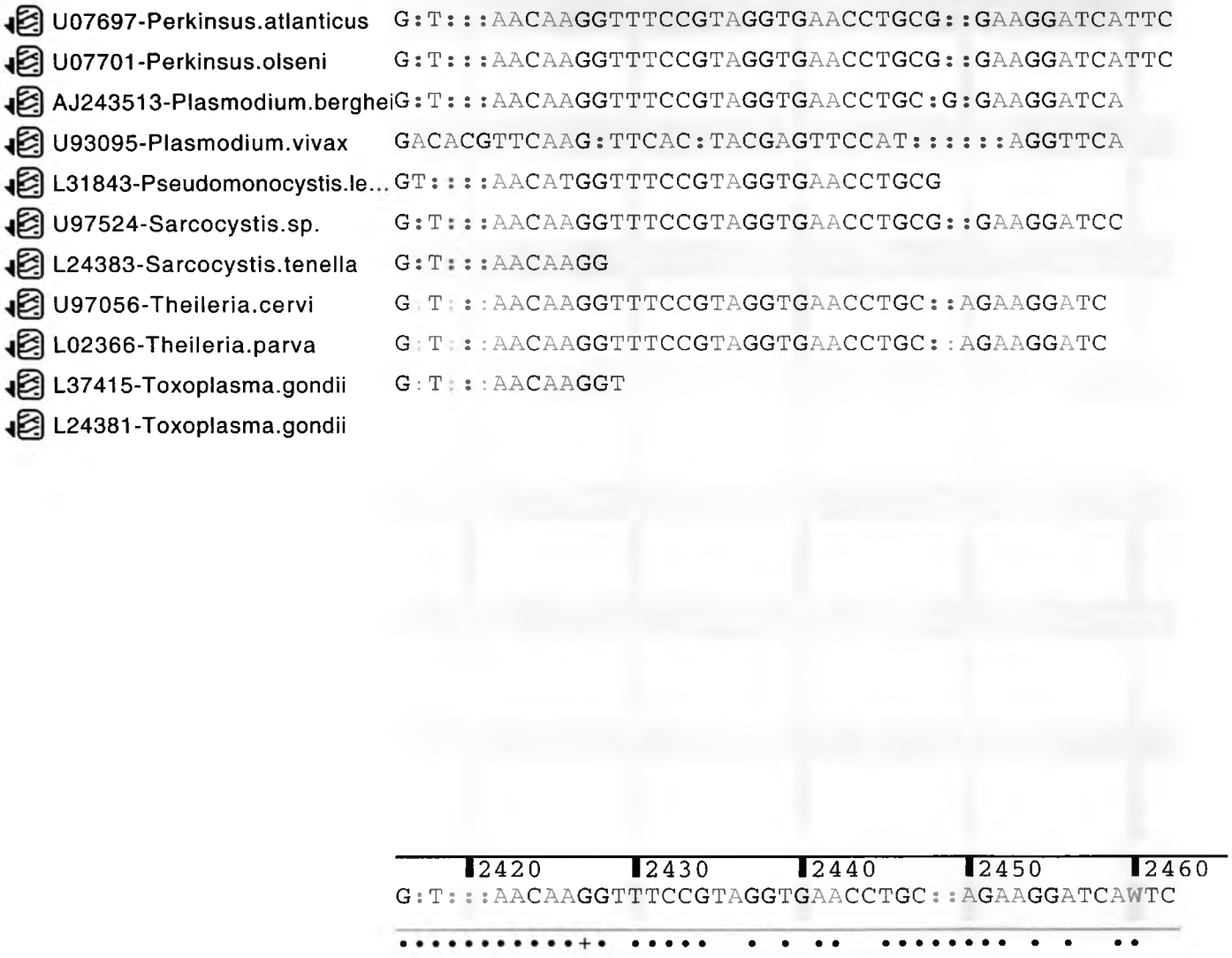
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AJ243513-Plasmodium.berghei G:T:::AACAAGGTTTCCGTAGGTGAACCTGC:G:GAAGGATCA
U93095-Plasmodium.vivax GACACGTTCAAG:TTCAC:TACGAGTTCCAT::::::AGGTTCA
L31843-Pseudomonocystis.le... GT::::AACATGGTTTCCGTAGGTGAACCTGCG
U97524-Sarcocystis.sp. G:T:::AACAAGGTTTCCGTAGGTGAACCTGCG::GAAGGATCC
L24383-Sarcocystis.tenella G:T:::AACAAGG
U97056-Theileria.cervi G:T:::AACAAGGTTTCCGTAGGTGAACCTGC::AGAAGGATC
L02366-Theileria.parva G:T:::AACAAGGTTTCCGTAGGTGAACCTGC::AGAAGGATC
L37415-Toxoplasma.gondii G:T:::AACAAGGT
L24381-Toxoplasma.gondii
2420
2430
2440
2450
2460
G:T:::AACAAGGTTTCCGTAGGTGAACCTGC::AGAAGGATCAWTC

APPENDIX D

SMITH ET AL., 1999 SEQUENCE
AT PRIMER SITE

Smith et al., 1999:

Xenopus seq. 5'- CCG TAG GTG AAC CTG CGG AAG -3'
Hepatozoon seq. CGG TAG GTG AAC CTG CGG AAG
ITS-7 forward CGG TAG GTG AAC CTG CGG AAG

Xenopus seq. 5'- GCA TCG ATG AAG GAC GCA GC -3'
Hepatozoon seq. GCA TCG ATG AAG GAC GCA GC
ITS-8 reverse GCA TCG ATG AAG GAC GCA GC

Sequence obtained from NCBI Gen Bank.
Xenopus accession number X02995
Hepatozoon accession number AF110241

APPENDIX E

ALIGNMENT OF TURTLE SEQUENCES

Appendix E


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kinostern.odoratum TGACTACCAAAAGCTCATGTAGAAGCCCCAATTGCAGGCTCTATAATCCTAGCAGCTGTACTACTTAAACTAGGAG
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pseudemys.texana TGACTACCAAAAGCTCATGTAGAAGCCCCAATCGCAGGGTCAATAATCCTAGCAGCAGTACTACTTAAACTAGGGG
siebenrockiella.cr... TGACTACCAAAAGCTCATGTAGAAGCACCAGTCGCCGGATCCATAATCCTAGCAGCAGTACTGCTCAAACTAGGAG
trachemys.scripta TGACTACCAAAAGCTCATGTAGAAGCCCCAATCGCAGGATCAATAATCCTAGCAGCAGTACTACTCAAATTAGGGG
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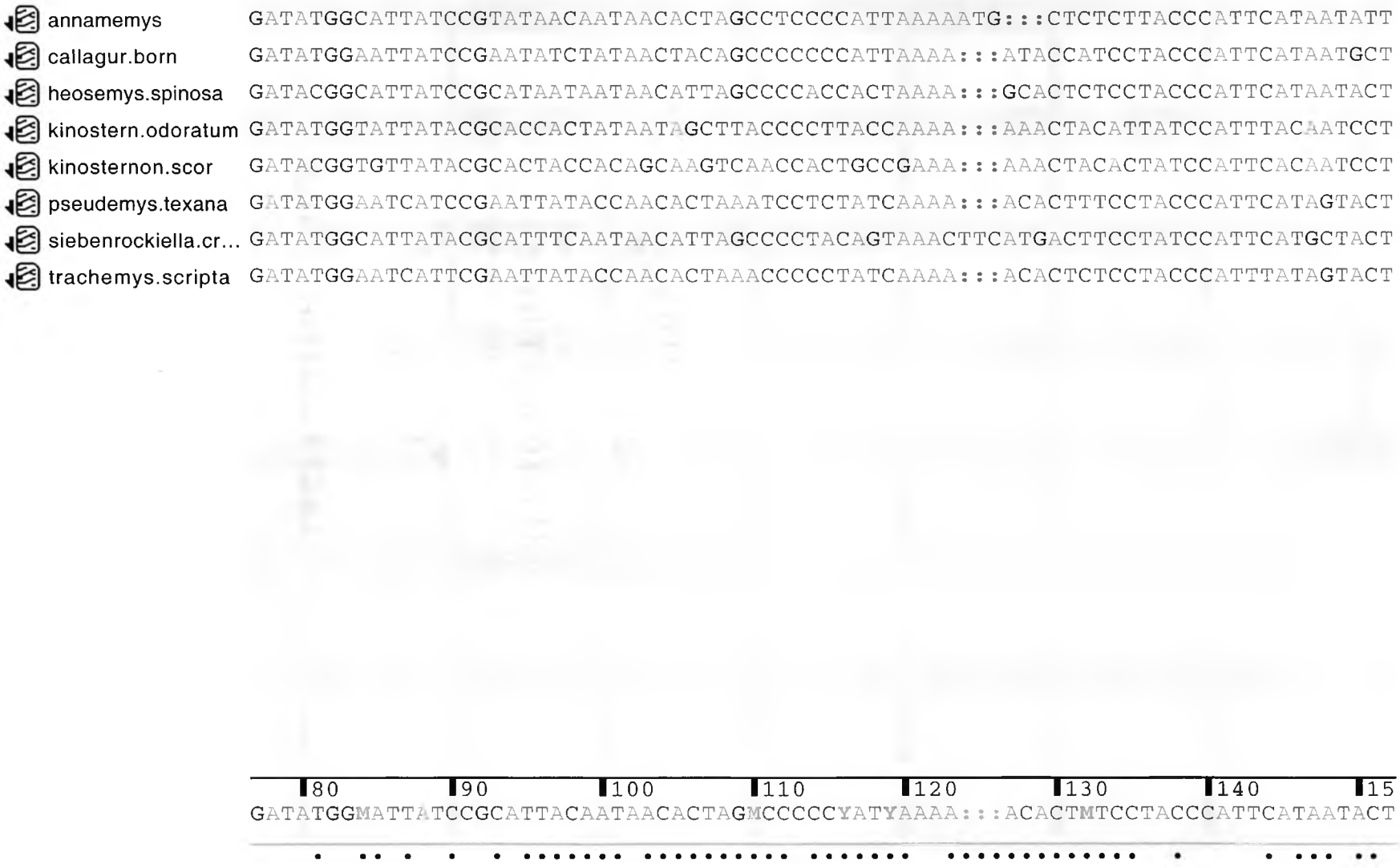
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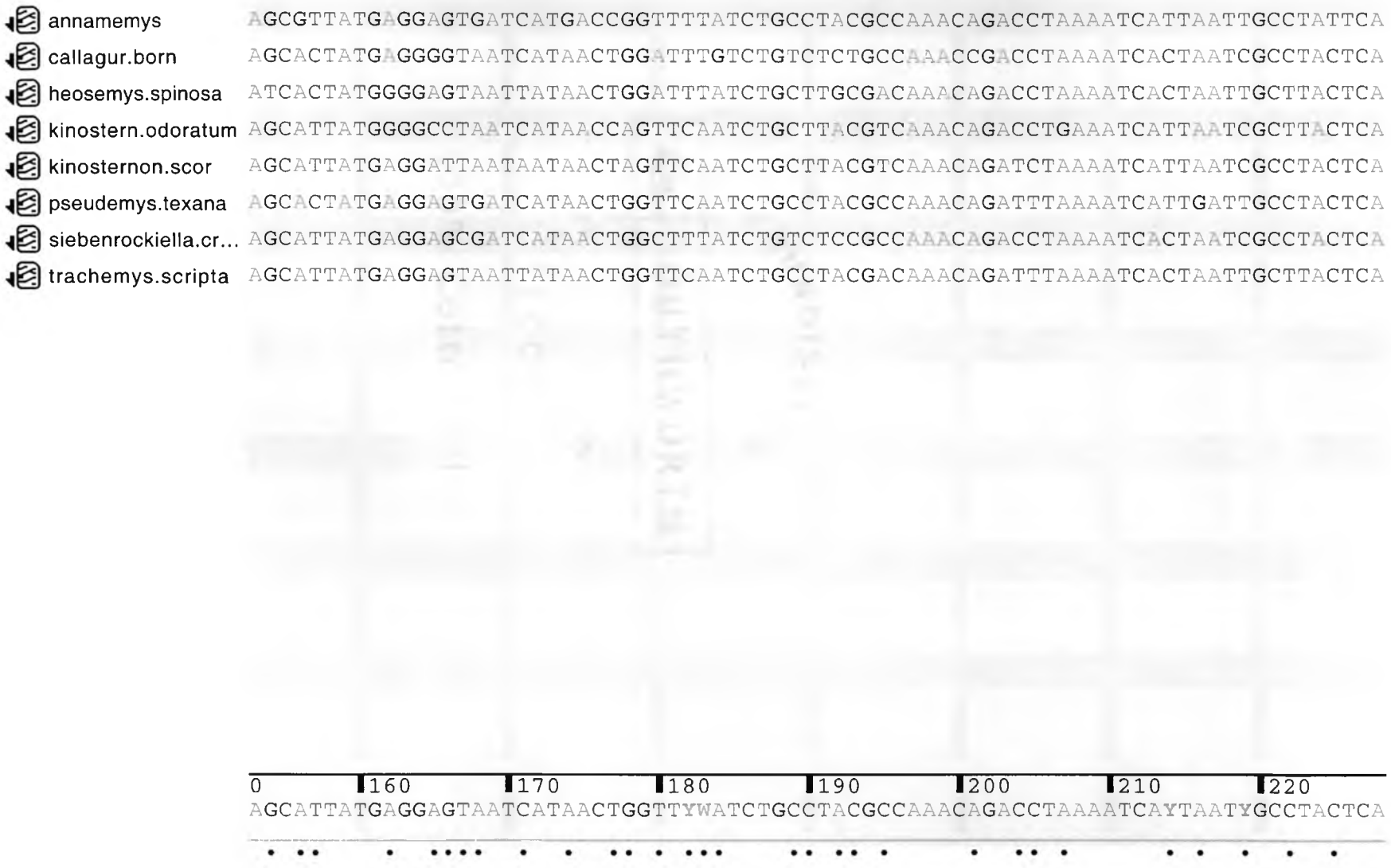

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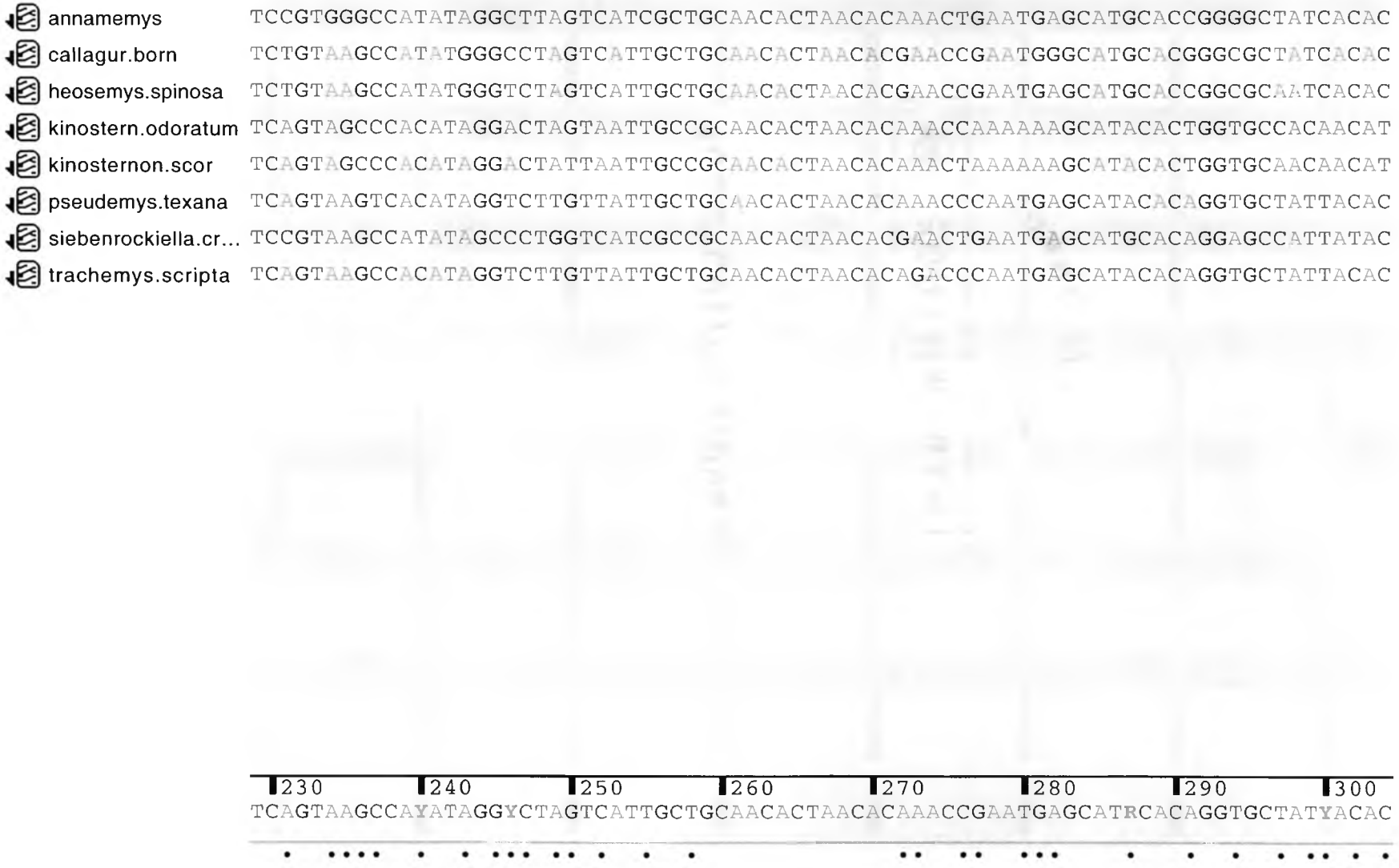

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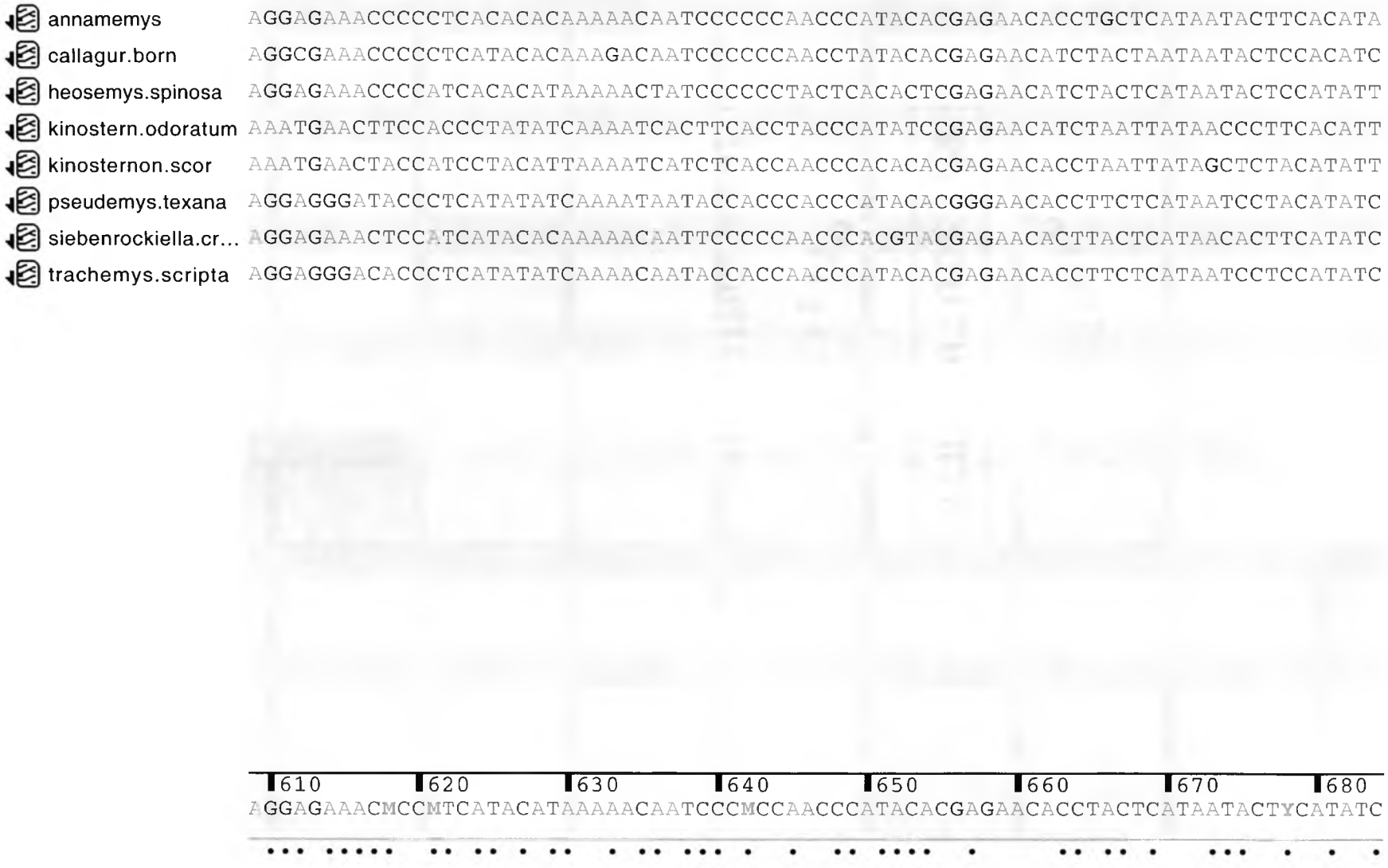
Appendix E (cont.)

Appendix E (cont.)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kinosternon.scor AAATGAACTACCATCCTACATTAAAATCATCTCACCAACCCACACACGAGAACACCTAATTATAGCTCTACATATT
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siebenrockiella.cr... AGGAGAAACTCCATCATACACAAAAACAATTCCCCCAACCCACGTACGAGAACACTTACTCATAACACTTCATATC
trachemys.scripta AGGAGGGACACCCTCATATATCAAAACAATACCACCAACCCATACACGAGAACACCTTCTCATAATCCTCCATATC
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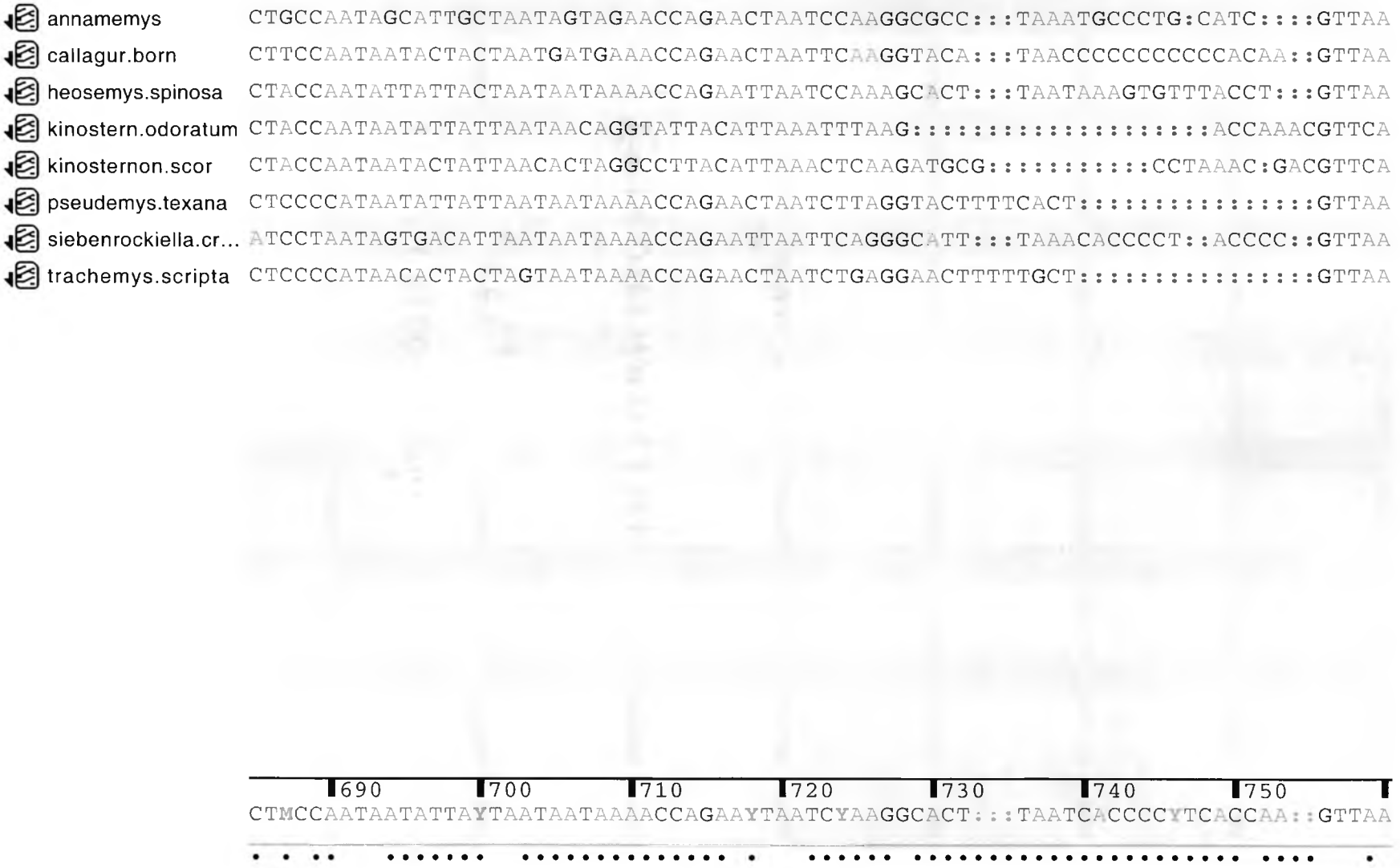
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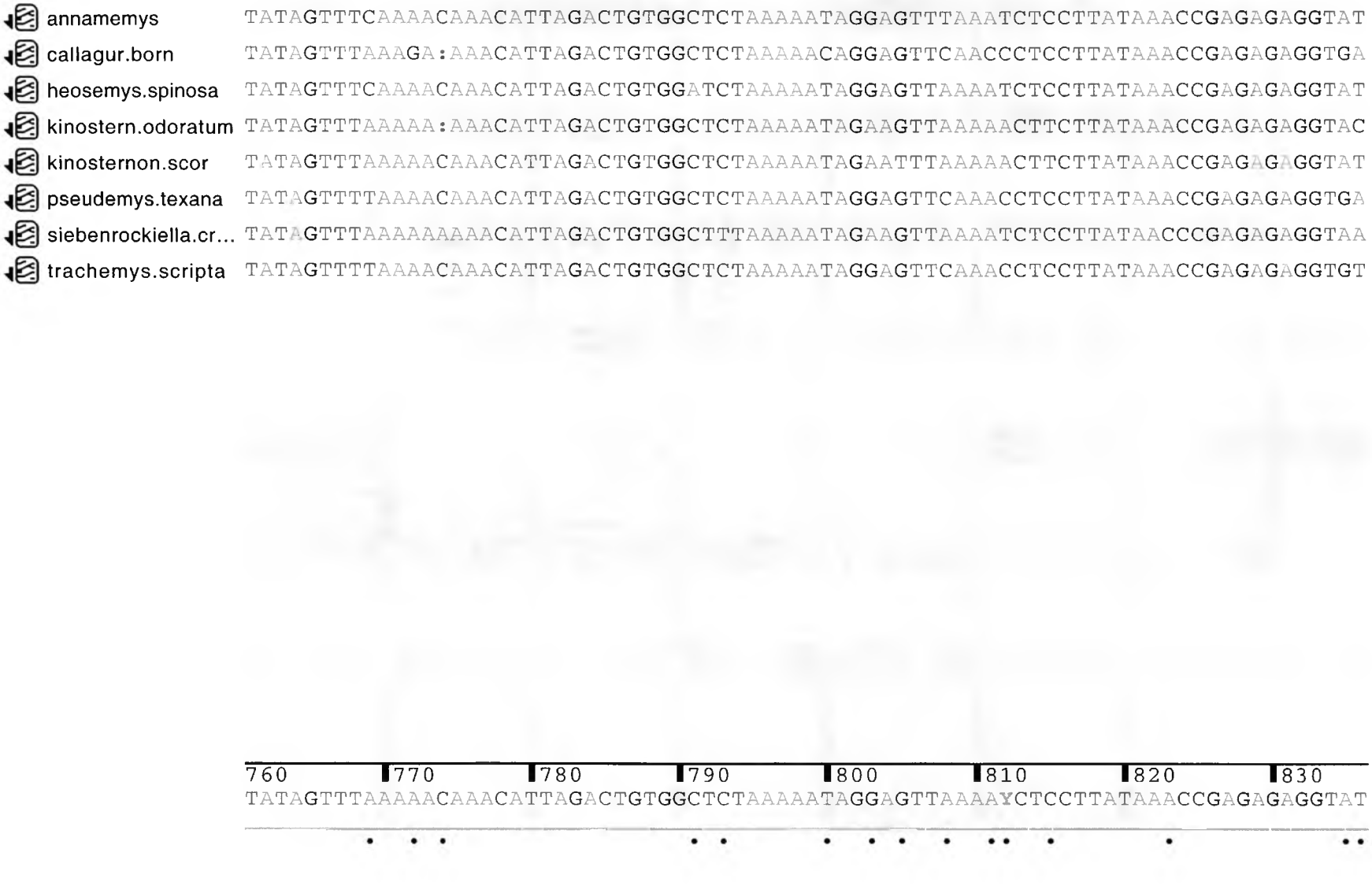

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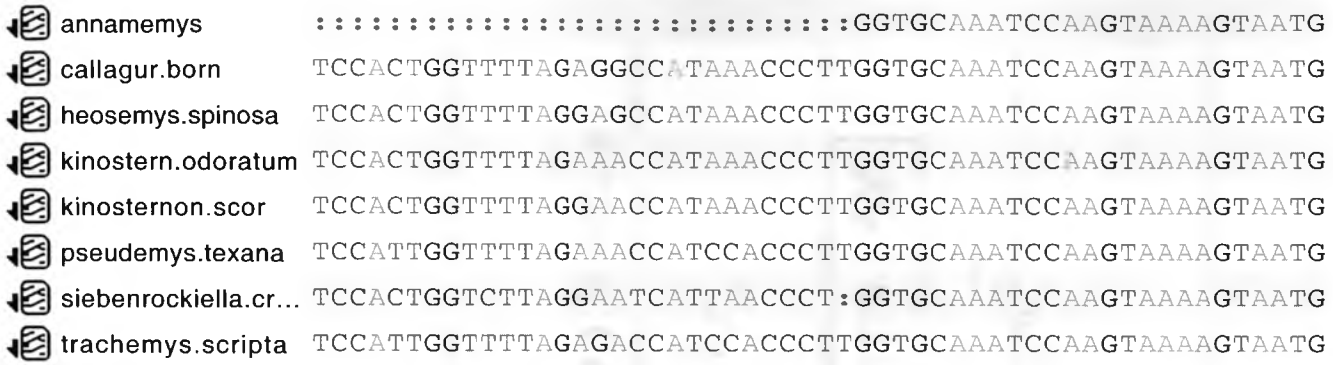
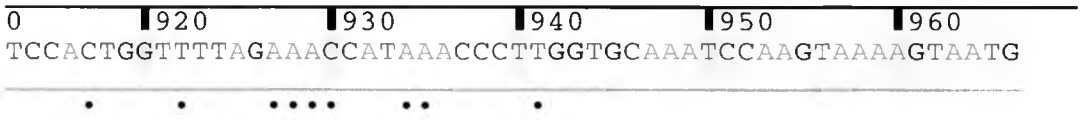
Appendix E (cont.)





APPENDIX F

PHYLOGENETIC ANALYSES

Dataset A (Phyla-level analyses):

- Maximum parsimony
  - Gaps as 5th base
    - Figure F-1. Most parsimonious tree 1
    - Figure F-2. Most parsimonious tree 2
    - Figure F-3. Bootstrap
    - Figure F-4. Jackknife
  - Gaps as missing data
    - Figure F-5. Strict consensus of 30 most parsimonious trees
    - Figure F-6. Majority rule of 30 most parsimonious trees
    - Figure F-7. Bootstrap
    - Figure F-8. Jackknife
- Distance-Neighbor Joining
  - Figure F-9. Neighbor Joining tree-cladogram
  - Figure F-10. Neighbor Joining tree-phylogram
  - Figure F-11. Bootstrap
  - Figure F-12. Jackknife
- Maximum Likelihood
  - Figure F-13. ML tree Model 1
  - Figure F-14. Bootstrap
  - Figure F-15. ML tree Model 2
  - Figure F-16. Bootstrap

Dataset D (Turtle genera analyses):

Maximum parsimony

Gaps as 5th base

Figure F-49. Most parsimonious tree

Figure F-50. Bootstrap & Jackknife

Gaps as missing data

Figure F-51. Most parsimonious

Figure F-52. Bootstrap & Jackknife

Distance-Neighbor Joining

Figure F-53. Neighbor Joining tree-cladogram

Figure F-54. Neighbor Joining tree-phylogram

Figure F-55. Bootstrap & Jackknife

Maximum Likelihood

Figure F-56. ML tree Model 1

Figure F-57. Bootstrap

For all references to accepted NCBI systematics, see Table 8. *Cryptosporidium* species were abbreviated as *Crypto*. For all *Haemogregarina* sp. taxon labels, the MF number refers to the voucher specimen number, and does not represent a GenBank accession number. *Haemogregarina* sp. hosts are abbreviated as: HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonorianse*, TSE=*Trachemys scripta elegans*.

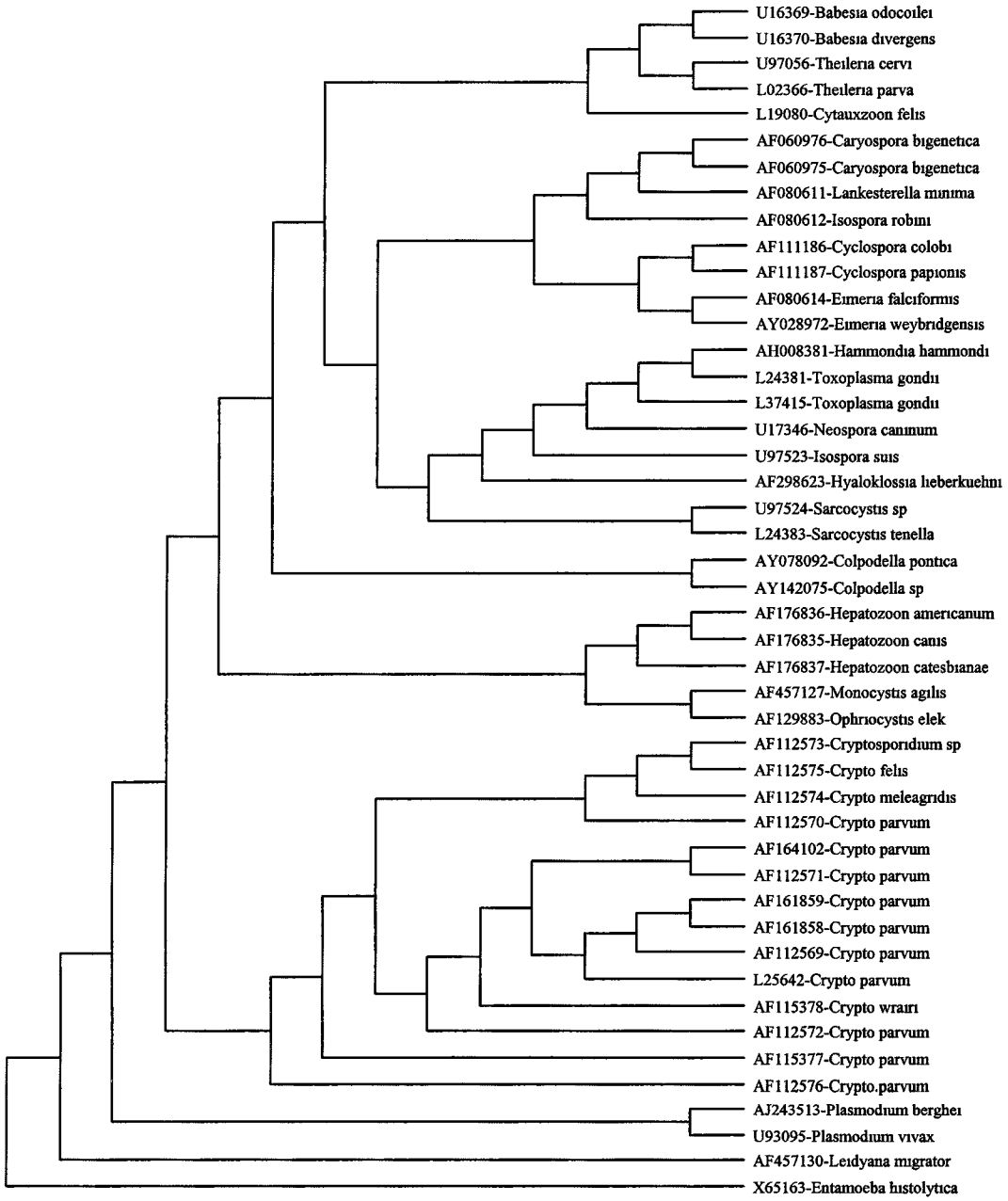


Figure F-1 Dataset A: Tree 1 of only 2 most parsimonious trees. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. Gaps were treated as 5th base and nucleotides weighted equally. The 2 most parsimonious trees had a TL=2749, CI=0.536, RI=0.698, and RC=0.374. The two most parsimonious topologies differ only in the placement of the *Cryptosporidium parvum* isolates L25642 and AF112569 within the monophyletic Cryptosporidiidae. *Leidyana migrator* failed to demonstrate monophyly within the Eugregarinida order, and the *Isospora suis* failed to demonstrate monophyly within the Eimeriidae with the other *Isospora* isolate. *Lankesterella* shows monophyly within the Eimeriidae and fails to exhibit placement as its own family within Eucoccidiorida. *Cytauxzoon* fails to show monophyly within the Theileriidae.

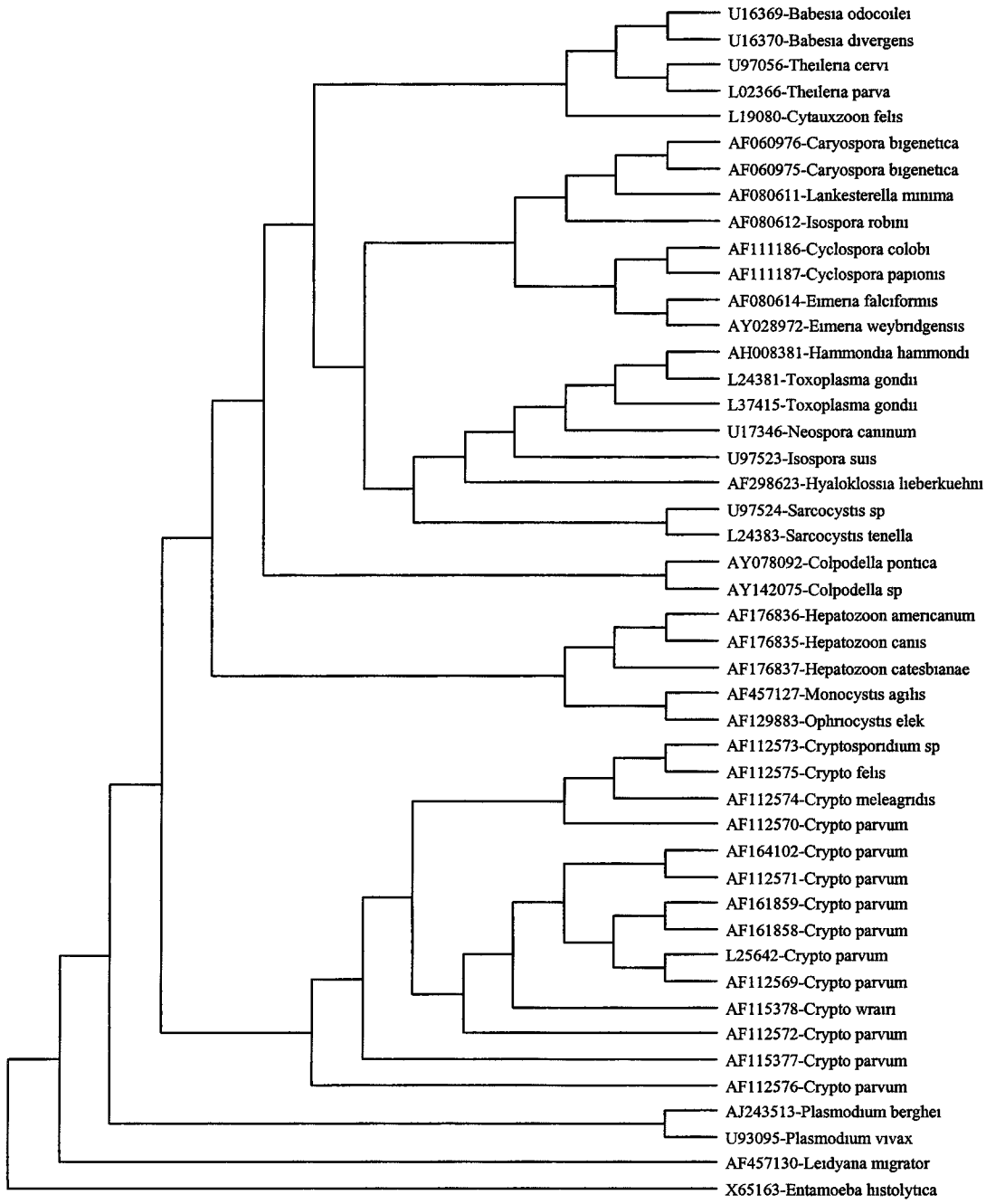


Figure F-2. Dataset A. Tree 2 of only 2 most parsimonious trees. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. Gaps were treated as 5th base and nucleotides weighted equally. The 2 most parsimonious trees had a TL=2749, CI=0.536, RI=0.698, and RC=0.374. The 2 most parsimonious trees had a TL=2749, CI=0.536, RI=0.698, and RC=0.374. The two most parsimonious topologies differ only in the placement of the *Cryptosporidium parvum* isolates L25642 and AF112569 within the monophyletic Cryptosporidiidae. *Leidyana migrator* failed to demonstrate monophyly within the Eugregarinida order, and the *Isospora suis* failed to demonstrate monophyly within the Eimeriidae with the other *Isospora* isolate. *Lankesterella* shows monophyly within the Eimeriidae and fails to exhibit placement as its own family within Eucoccidiorida. *Cytauxzoon* fails to show monophyly within the Theileriidae.

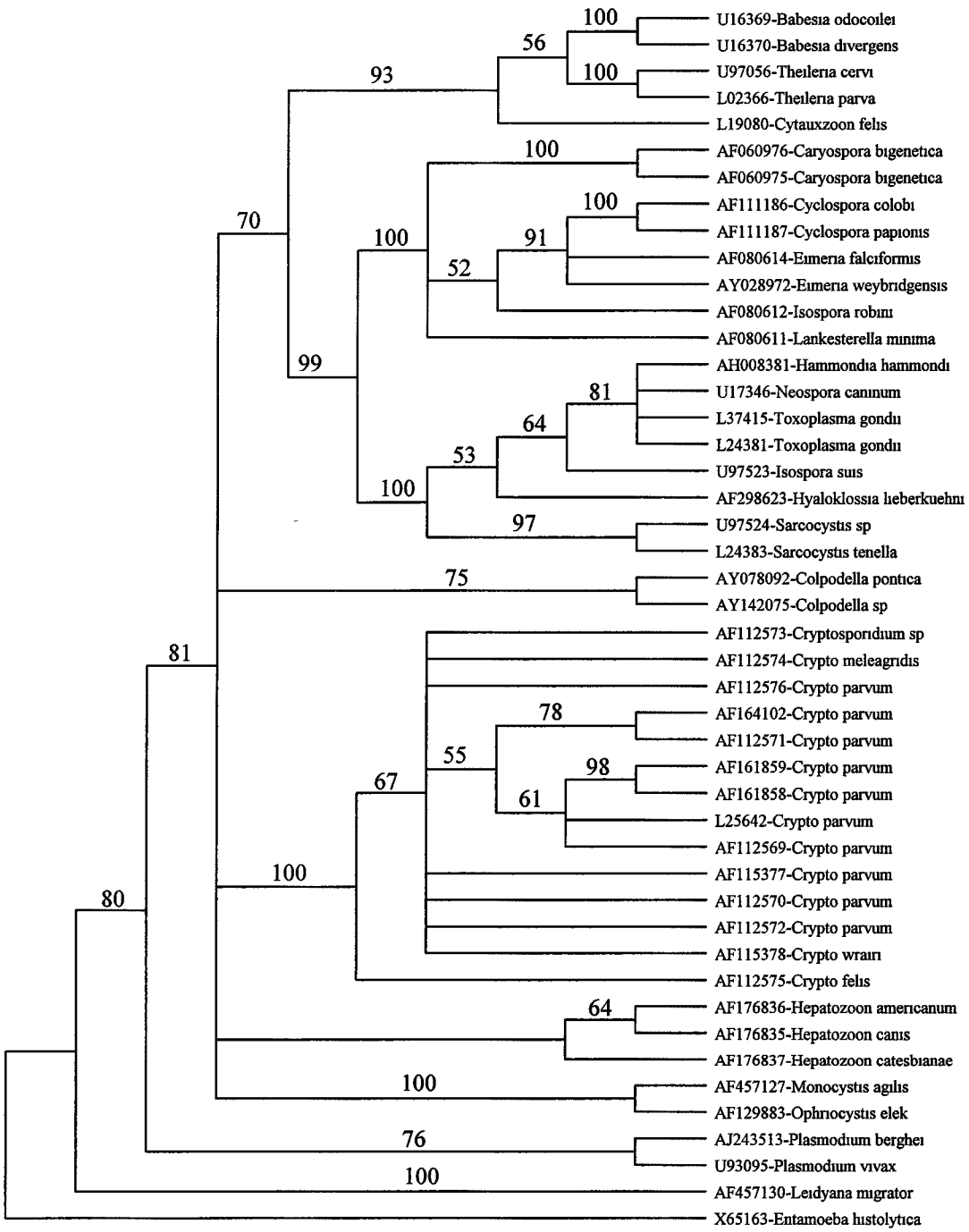


Figure F-3. Dataset A: Bootstrap consensus tree by maximum parsimony with bootstrap values <50% support not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum parsimony, a bootstrap heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. Gaps were treated as 5th base and nucleotides weighted equally. The class Gregarina fails to demonstrate monophyly, and the class Coccidia also fails to exhibit monophyly. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiididae) show strong support as monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in Figures F-1 and F-2.

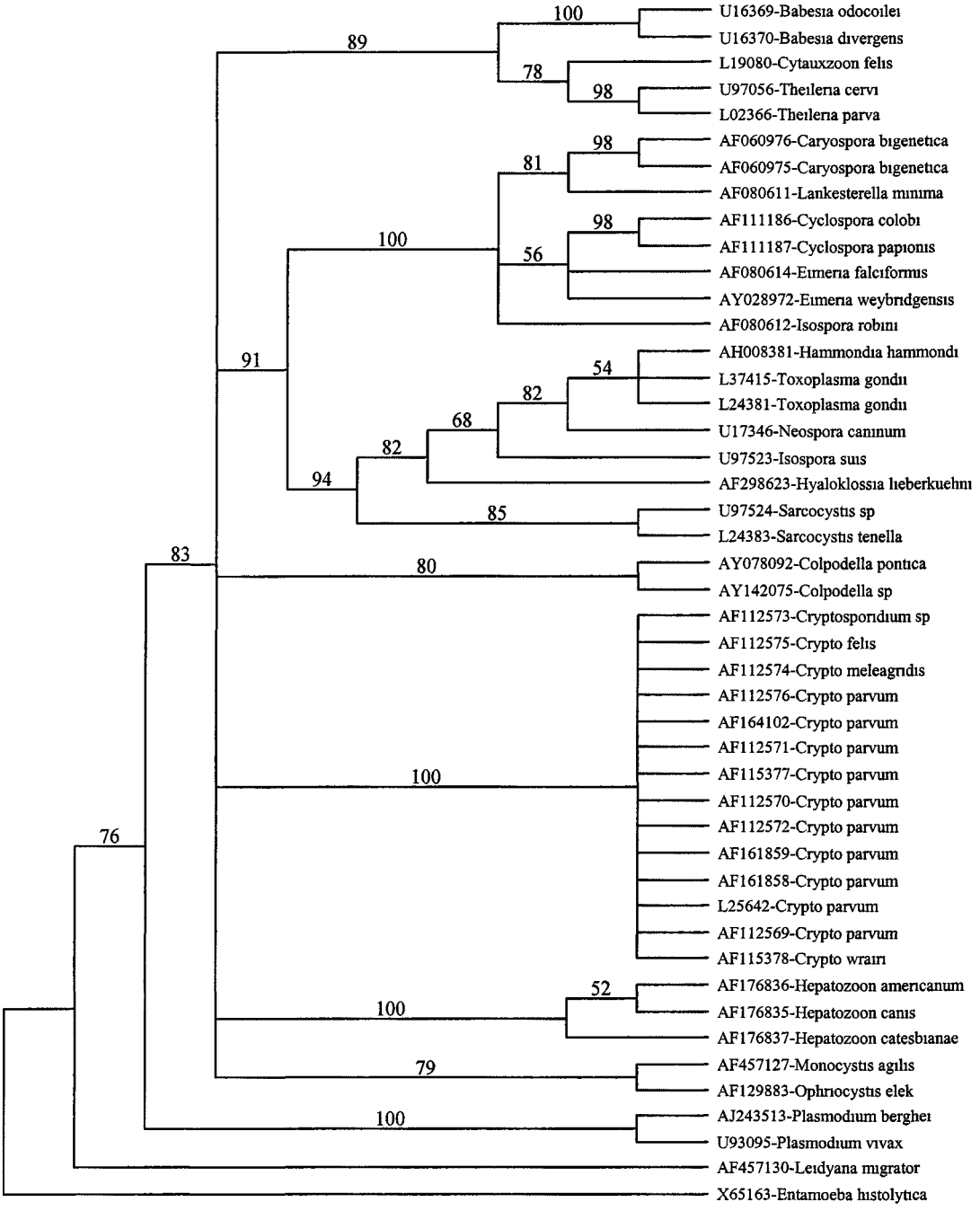


Figure F-4. Dataset A: Jackknife consensus tree by maximum parsimony with jackknife values <50% support not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum parsimony, a jackknife heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. Gaps were treated as 5th base and nucleotides weighted equally. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiidae) show strong support as monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in Figures F-1, F-2 and F-3. The systematically established monophyly of Cytauxzoon within the Theileriidae shows stronger support than on the Bootstrap Consensus from Figure F-3.

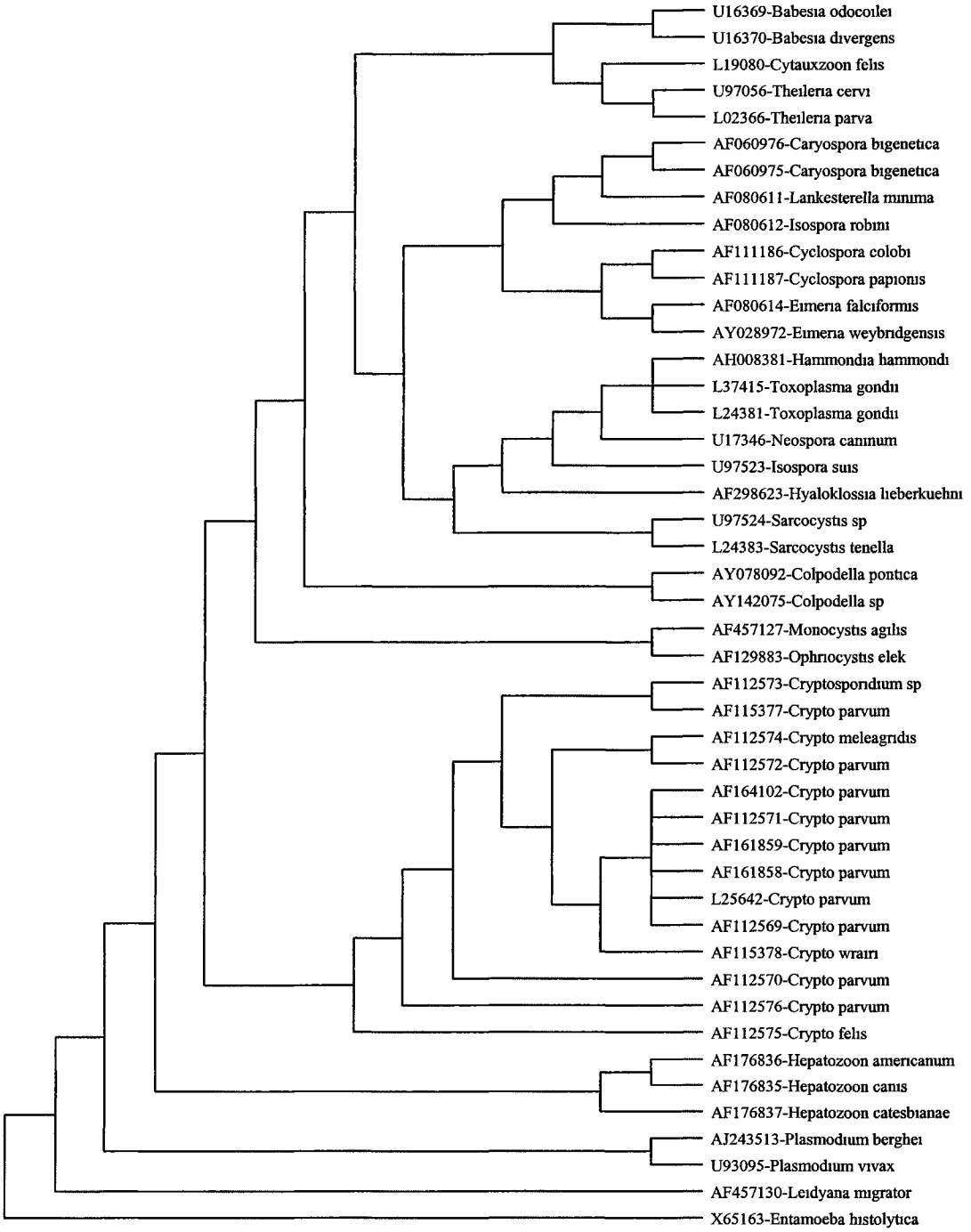


Figure F-5. Dataset A: Strict consensus of 30 most parsimonious trees with gaps treated as missing and nucleotides weighted equally. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. The 30 most parsimonious trees had a TL=1796, CI=0.579, RI=0.734, and RC=0.425. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiididae) represent monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in Figures F-1 through F-4 with gaps treated as a 5th base under maximum parsimony criterion. The systematically accepted Eucoccidiorida (Haemogregarinidae, Lankesterellidae, and Colpodellidae) fails to exhibit monophyly, as well as the Eugregarinidae (Leidyanidae, Monocystidae, and Ophryocystidae) fails to demonstrate a monophyletic unit.

Figure F-6 Dataset A· Majority rule consensus of 30 most parsimonious trees with gaps treated as missing and nucleotides weighted equally. *Entamoeba histolytica* was set as outgroup to the Apicomplexa With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. The 30 most parsimonious trees had a TL=1796, CI=0 579, RI=0.734, and RC=0.425. The majority rule consensus demonstrates that the topology differences among the 30 most parsimonious trees lie within the Cryptosporidiidae. All other branches on this topology show 100% of the same topology for the 30 most parsimonious trees This topology differs to Figures F-1 and F-2 (gaps as 5th base) in the presence of monophyly of Theileridae, presence of polytomy with *Toxoplasma* and *Hammondia*, lack of monophyly within Eugregarinida, and minor differences within Cryptosporidiidae

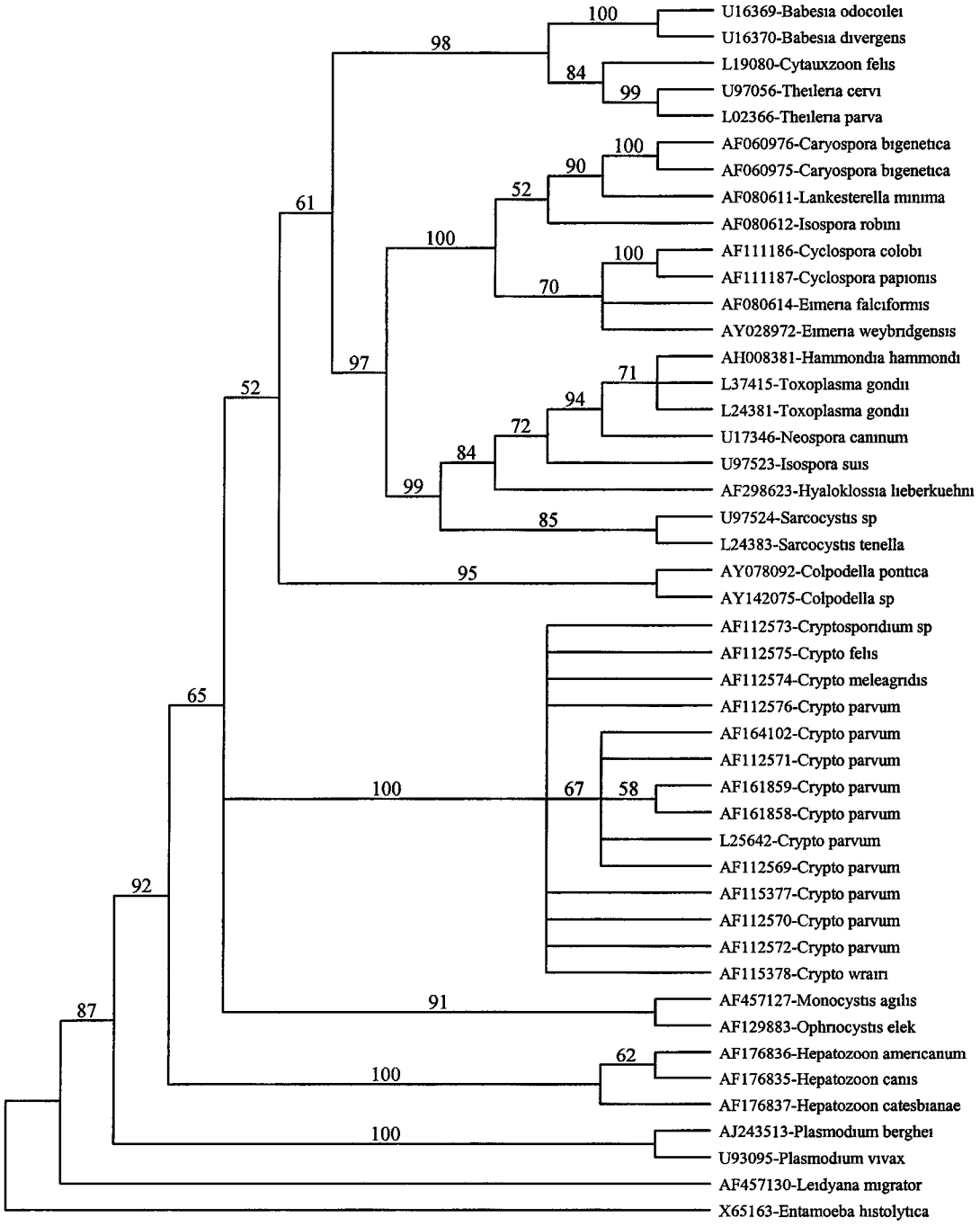


Figure F-7. Dataset A: Bootstrap consensus tree by maximum parsimony with bootstrap values <50% support not shown with gaps treated as missing and nucleotides weighted equally. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum parsimony, a bootstrap heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiididae) show strong support as monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in all previous topologies. This bootstrap consensus shows stronger support than the bootstrap consensus from Figure F-3 with gaps as missing data.

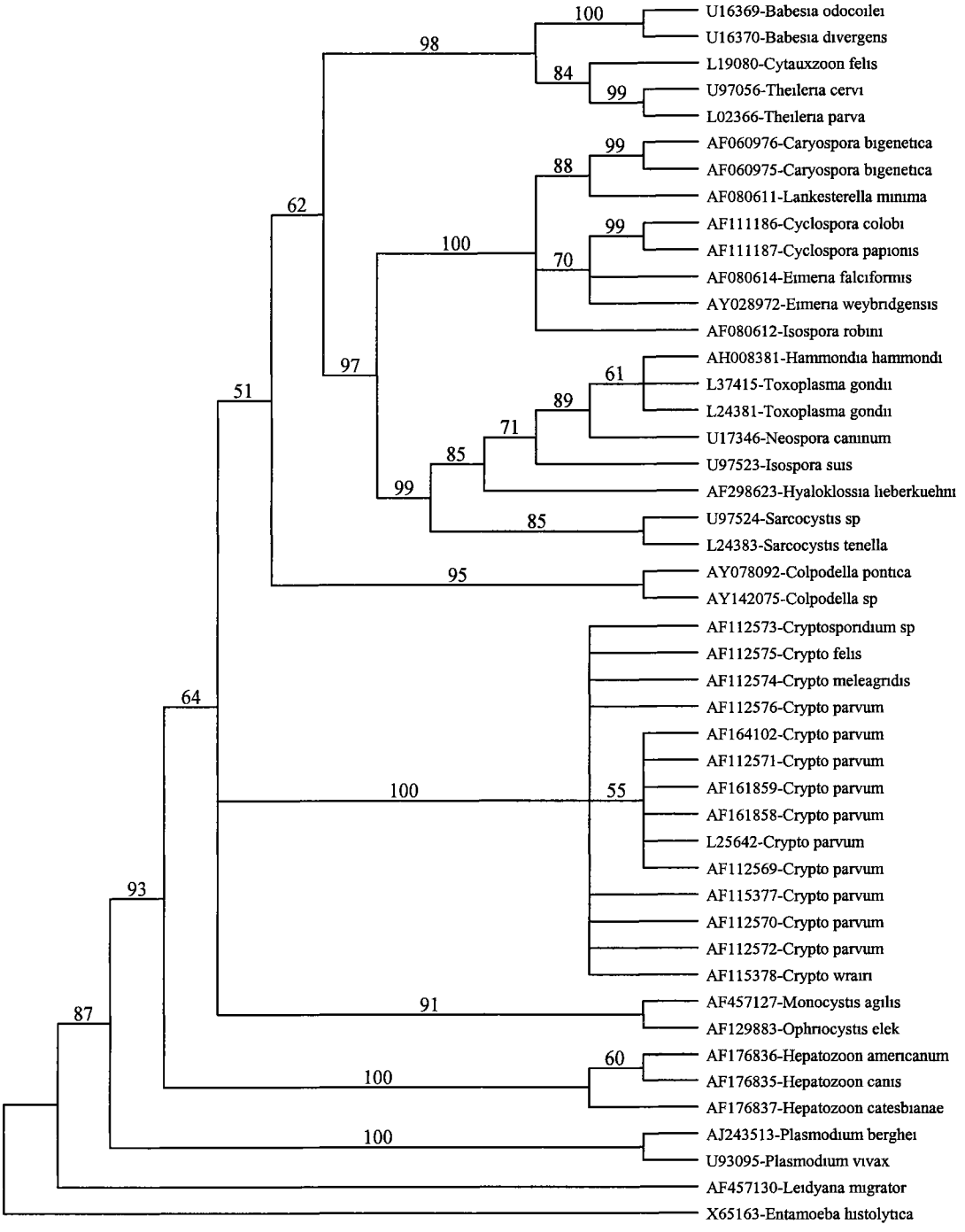


Figure F-8. Dataset A: Jackknife consensus tree by maximum parsimony with jackknife values <50% support not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum parsimony, a jackknife heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. Gaps were treated as missing and nucleotides weighted equally. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiididae) demonstrate monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in all previous topologies. This jackknife topology shows weaker support for classes within Apicomplexa however resolves polytomies compared to the jackknife topology from Figure F-4 with gaps as missing data.

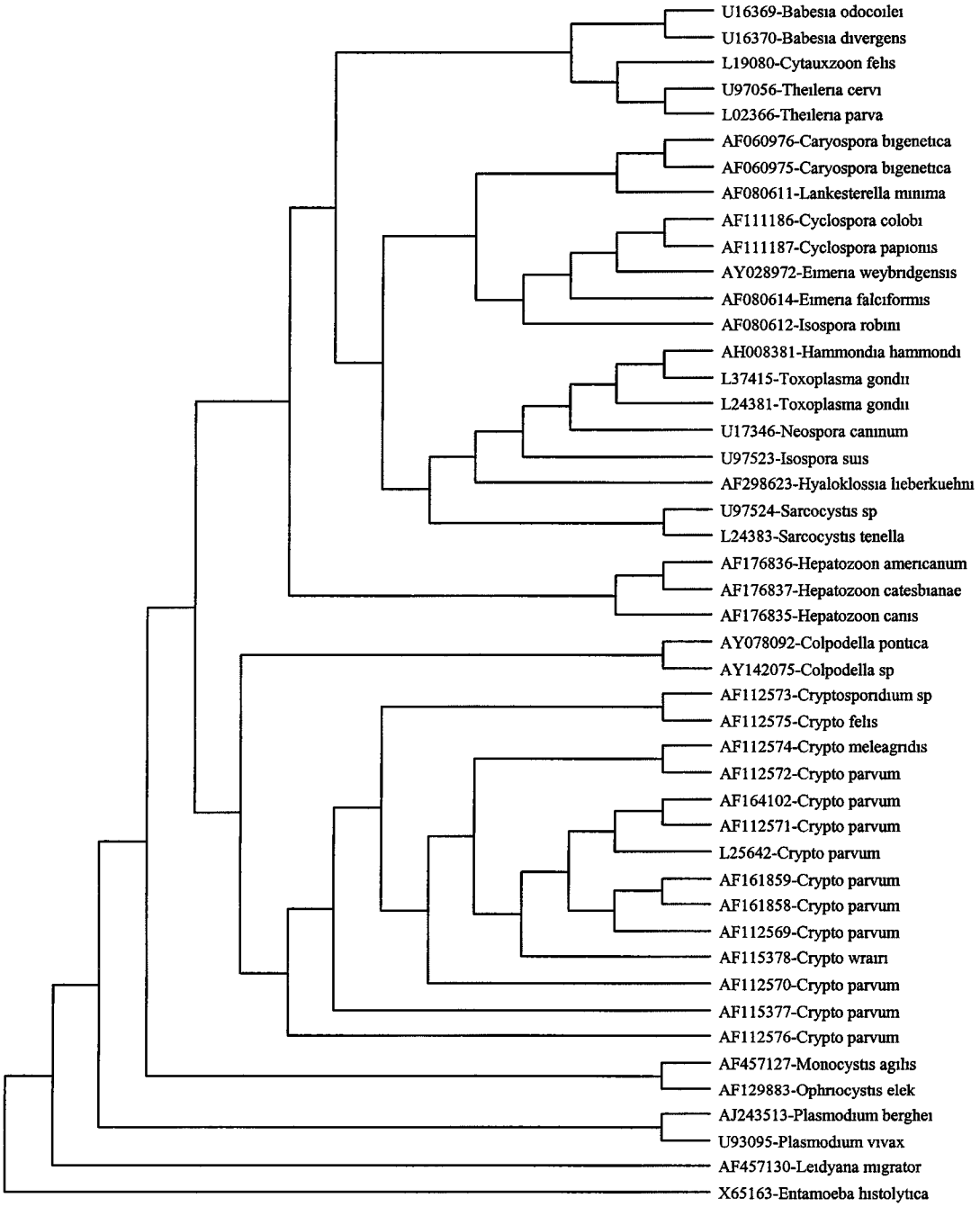


Figure F-9. Dataset A: Cladogram with optimality criterion set to distance with a neighbor joining search and Tajima Nei distance correction algorithm and nucleotides weighted equally. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. A neighbor joining search was performed for 1000 replicates by random stepwise addition with TBR branch swap method. The ME value is 1.64636. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiidae) demonstrate monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in all previous topologies. The Eugregarinida order fails to demonstrate monophyly, however the taxa within Cryptosporidiidae show resolution.

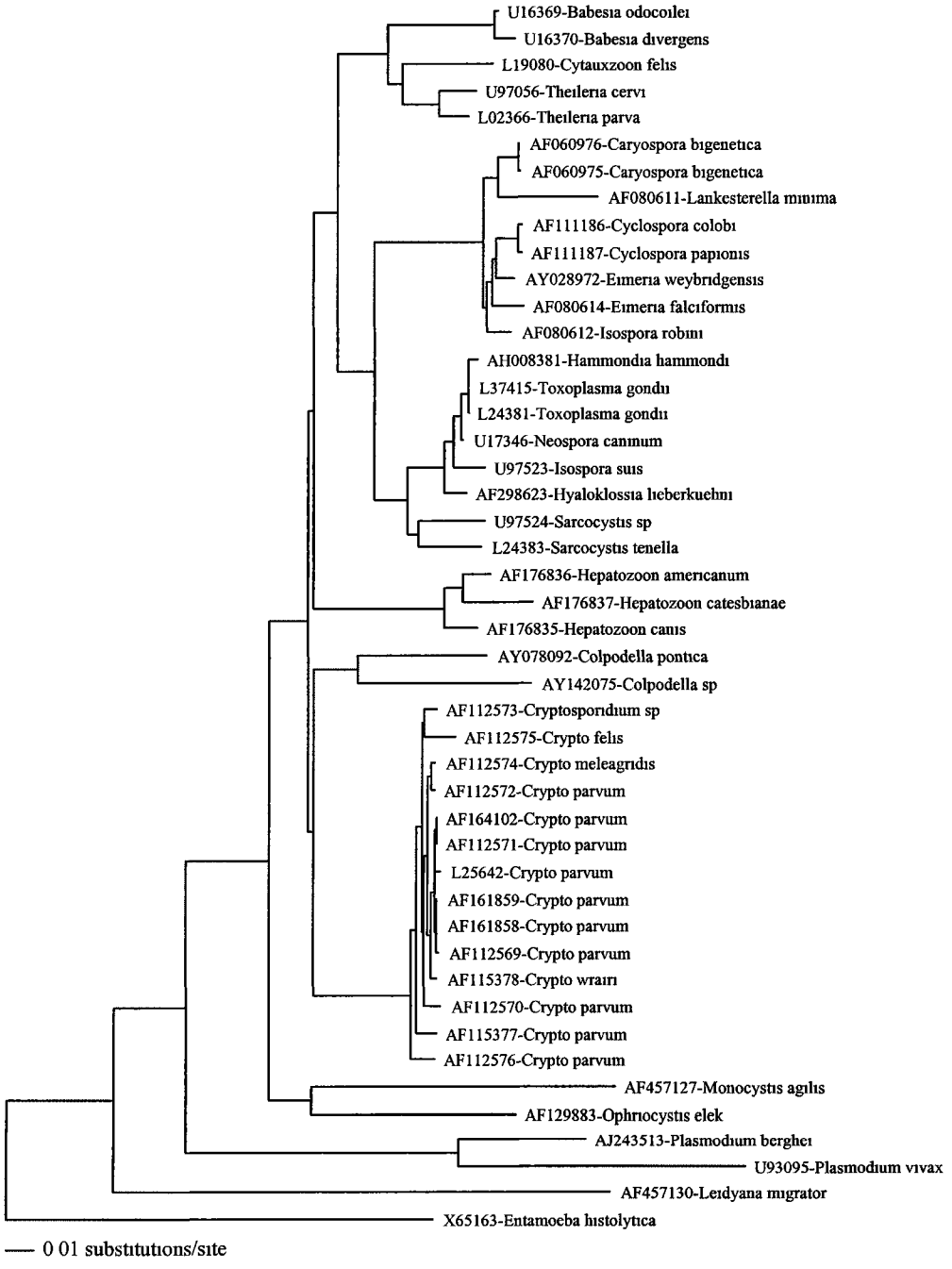


Figure F-10. Dataset A: Phylogram with optimality criterion set to distance with a neighbor joining search and Tajima Nei distance correction algorithm and nucleotides weighted equally. This is the same topology as in Figure F-9, however the number of substitutions per site are represented as a phylogram. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. A neighbor joining search was performed for 1000 replicates by random stepwise addition with TBR branch swap method. The ME value is 1.64636. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiidiidae) demonstrate monophyletic units respectively. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in all previous topologies, all of which show numerous substitutions per site in this topology. The Eugregarinida order fails to demonstrate monophyly, however the taxa within Cryptosporidiidae show resolution.

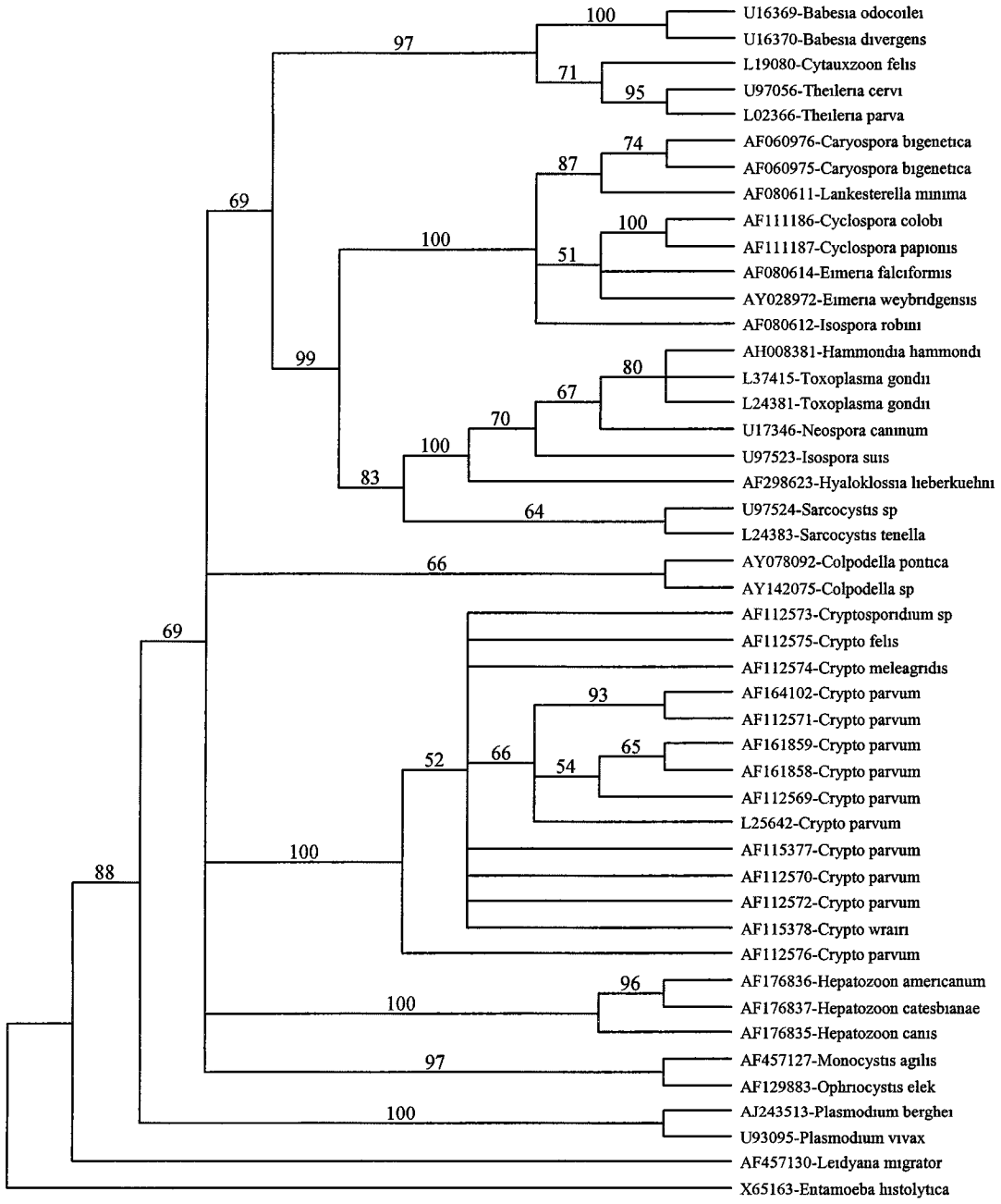


Figure F-11. Dataset A: Bootstrap consensus tree by neighbor joining with Tajima Nei distance correction algorithm and nucleotides weighted equally Bootstrap values <50% support are not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to distance, a bootstrap search was performed for 2500 replicates by neighbor joining method. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiidae) demonstrate monophyletic units respectively however show weak support. Eugregarinida fails to exhibit monophyly, and the Colpodellidae, Crytptosporidiidae, Haemogregarinidae, and Class Gregarina collapse into a polytomy. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in all previous topologies.

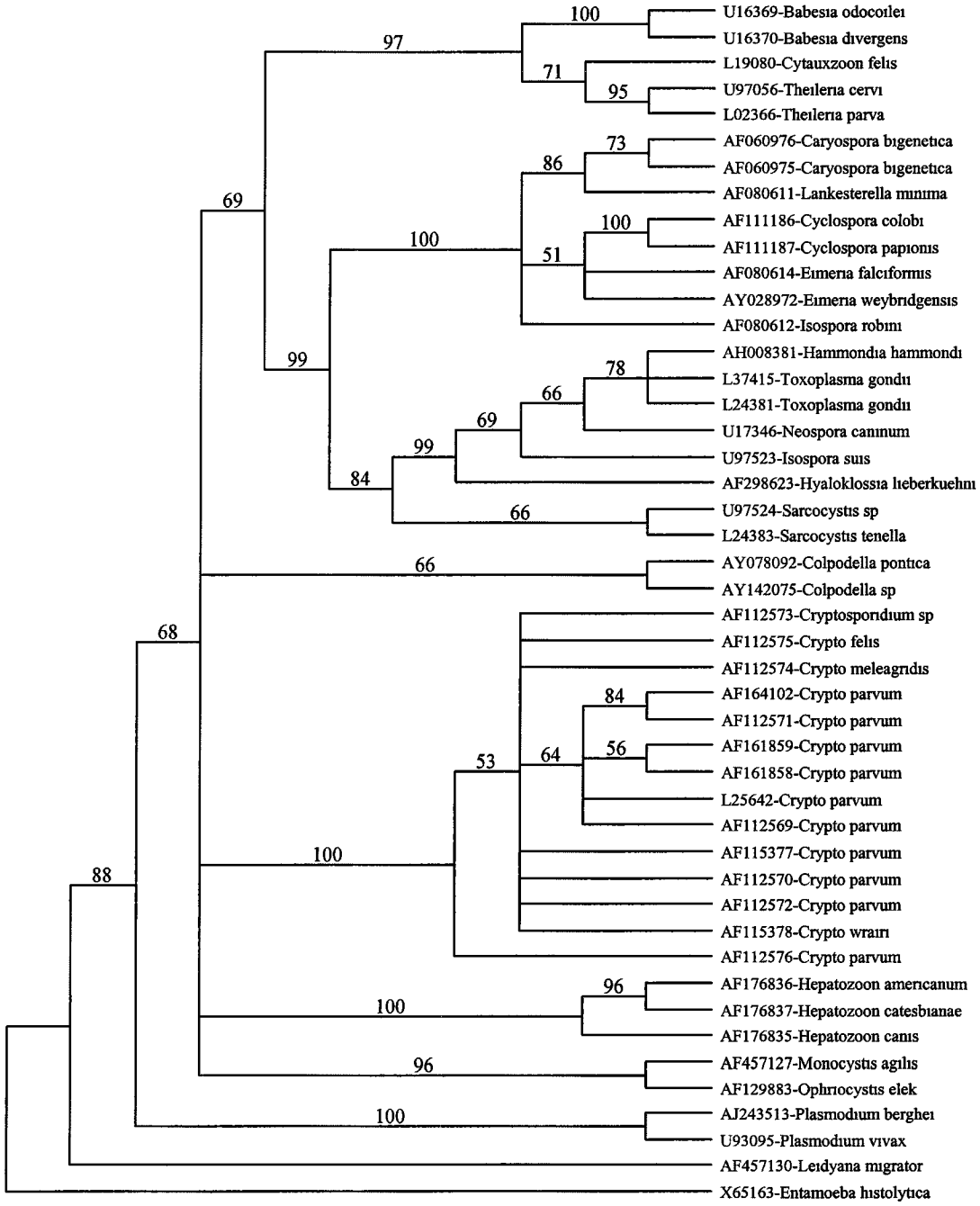


Figure F-12. Dataset A: Jackknife consensus tree by neighbor joining with Tajima Nei distance correction algorithm and nucleotides weighted equally. Jackknife values <50% support are not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to distance, a jackknife search was performed for 2500 replicates by neighbor joining method. The families within the Coccidia (Eimeriidae, Sarcocystidae, and Cryptosporiidae) demonstrate monophyletic units respectively however show weak support as also seen in Figure F-11. Eugregarinida fails to exhibit monophyly, and the Colpodellidae, Crytptosporidiidae, Haemogregarinidae, and Class Gregarina collapse into a polytomy. The placement of *Lankesterella*, *Isospora suis*, and *Leidyana* show the same systematic problems as seen in all previous topologies.

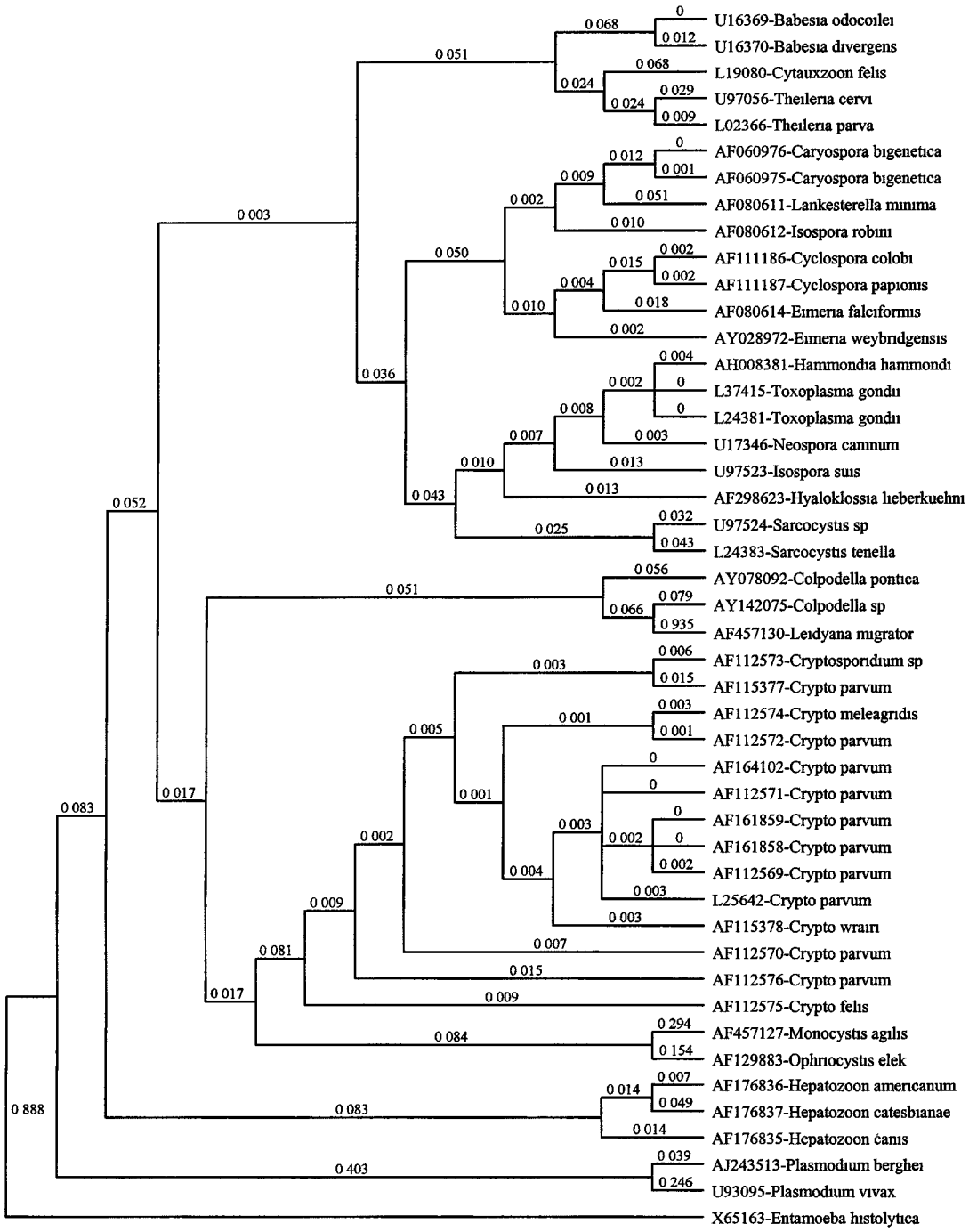


Figure F-13. Dataset A: Maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model GTR + I + G; base frequencies of A=0.3265, C=0.1652, G=0.2313, T=0.2771; substitution rate matrix of A-C=1.2767, A-G=2.5466, A-T=1.8424, C-G=0.8904, C-T=4.8326, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.3531. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum likelihood, a heuristic search was performed for by with TBR branch swap method and ran until completion. Nucleotides weighted equally. The –ln L score is 9273.59391. The Cryptosporidiidae, Colpodellidae, Monocystidae, and Ophryocystidae form a monophyletic clade that disagrees systematically. *Leidyana* forms a monophyletic clade with *Colpodella* alternatively to its systematic order Eugregarinida. *Isospora suis* and *Lankesterella* continue to disagree with systematic position.

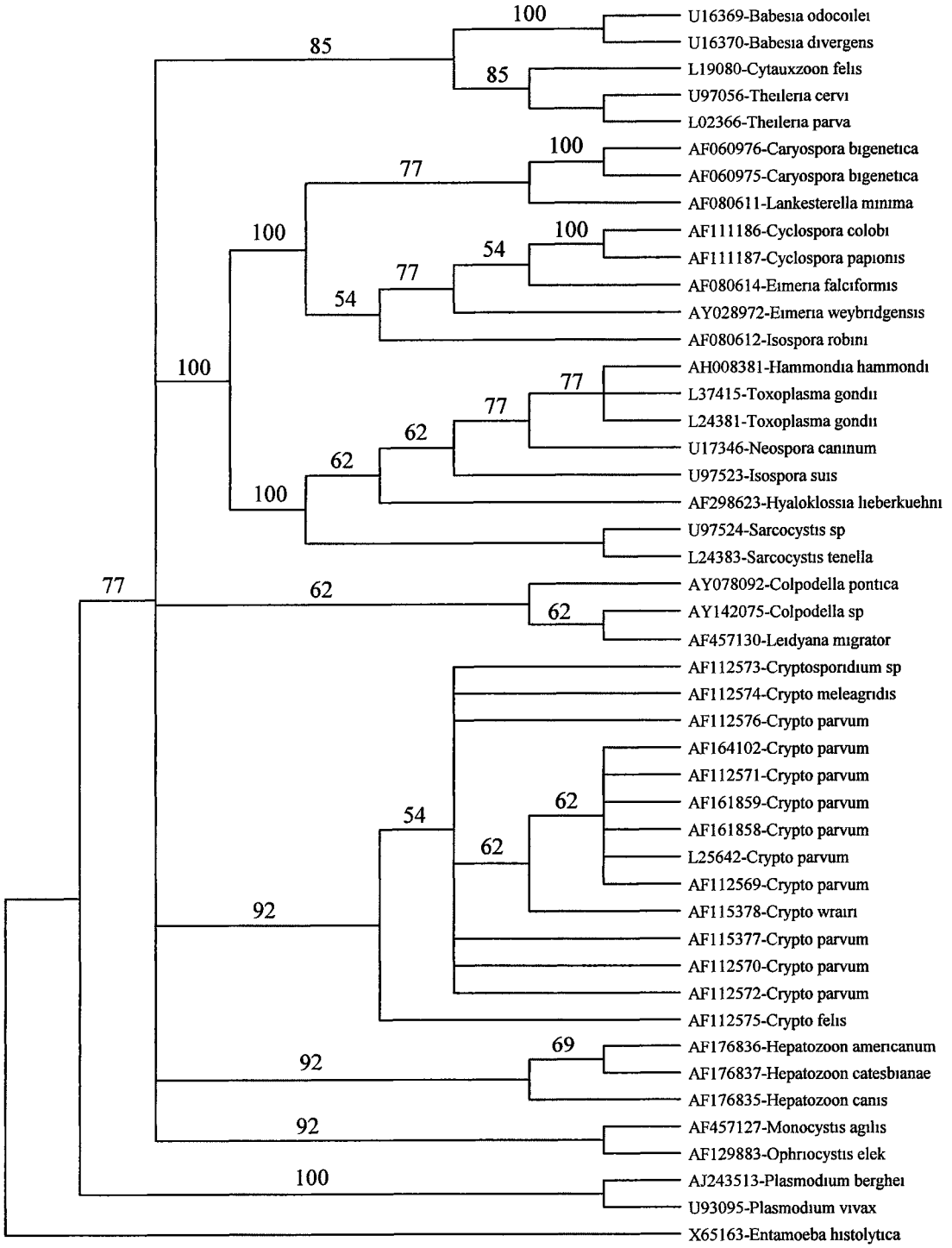


Figure F-14. Dataset A: Bootstrap consensus for maximum likelihood with branch lengths shown above branches using Modeltest parameters. The parameters included model GTR + I + G; base frequencies of A=0.3265, C=0.1652, G=0.2313, T=0.2771; substitution rate matrix of A-C=1.2767, A-G=2.5466, A-T=1.8424, C-G=0.8904, C-T=4.8326, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.3531. Bootstrap values <50% support are not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum likelihood, a bootstrap search was performed for 2500 replicates by neighbor joining method.

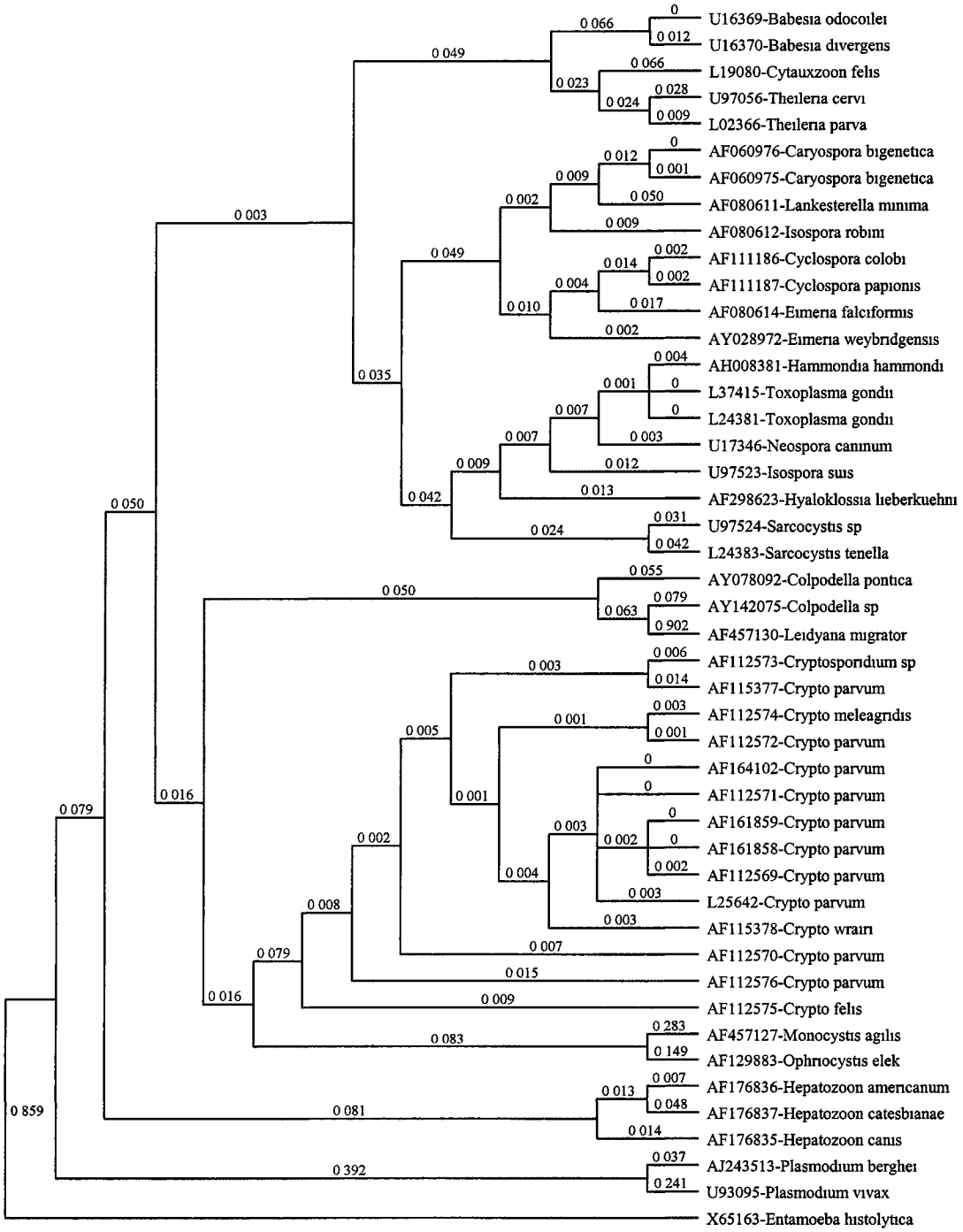


Figure F-15. Dataset A: Maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model GTR + I + G; base frequencies of A=0.3263, C=0.1647, G=0.2311, T=0.2778; substitution rate matrix of A-C=1.2873, A-G=2.5613, A-T=1.8402, C-G=0.8976, C-T=4.8366, and G-T=1.0000; ASRV invariable sites = 0.1126 and variable sites with a gamma distribution of 0.4359. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum likelihood, a heuristic search was performed for by with TBR branch swap method and ran until completion. Nucleotides weighted equally. The –ln L score is 9271.50200. This maximum likelihood model compared to Figure F-14 (different Modeltest parameters) is identical in topology, however the branch lengths are slightly different.

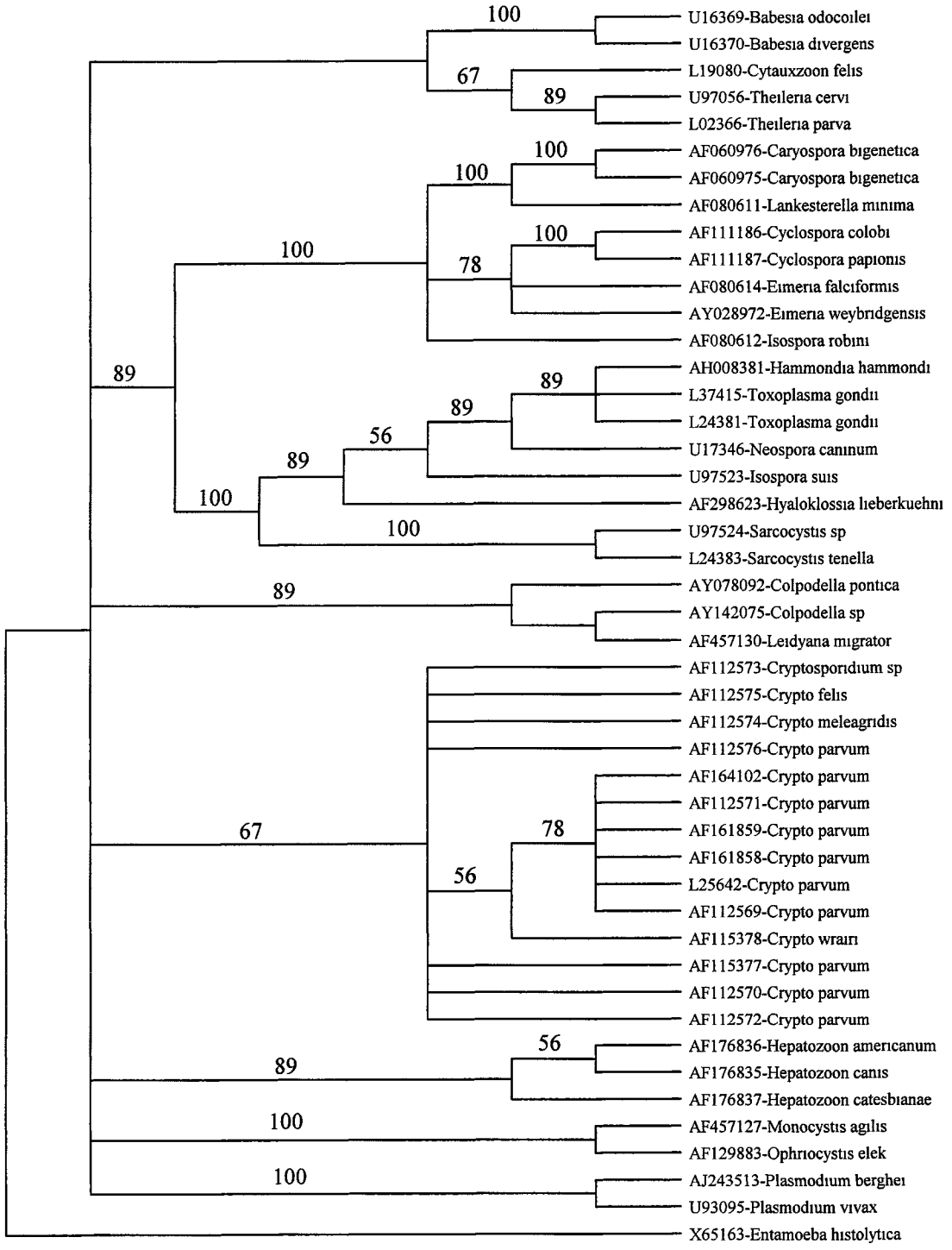


Figure F-16. Dataset A: Bootstrap consensus of maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model GTR + I + G; base frequencies of A=0.3263, C=0.1647, G=0.2311, T=0.2778; substitution rate matrix of A-C=1.2873, A-G=2.5613, A-T=1.8402, C-G=0.8976, C-T=4.8366, and G-T=1.0000; ASRV invariable sites = 0.1126 and variable sites with a gamma distribution of 0.4359. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. Bootstrap values <50% support are not shown. With optimality criteria set to maximum likelihood, a bootstrap search was performed for 2500 replicates by neighbor joining method.

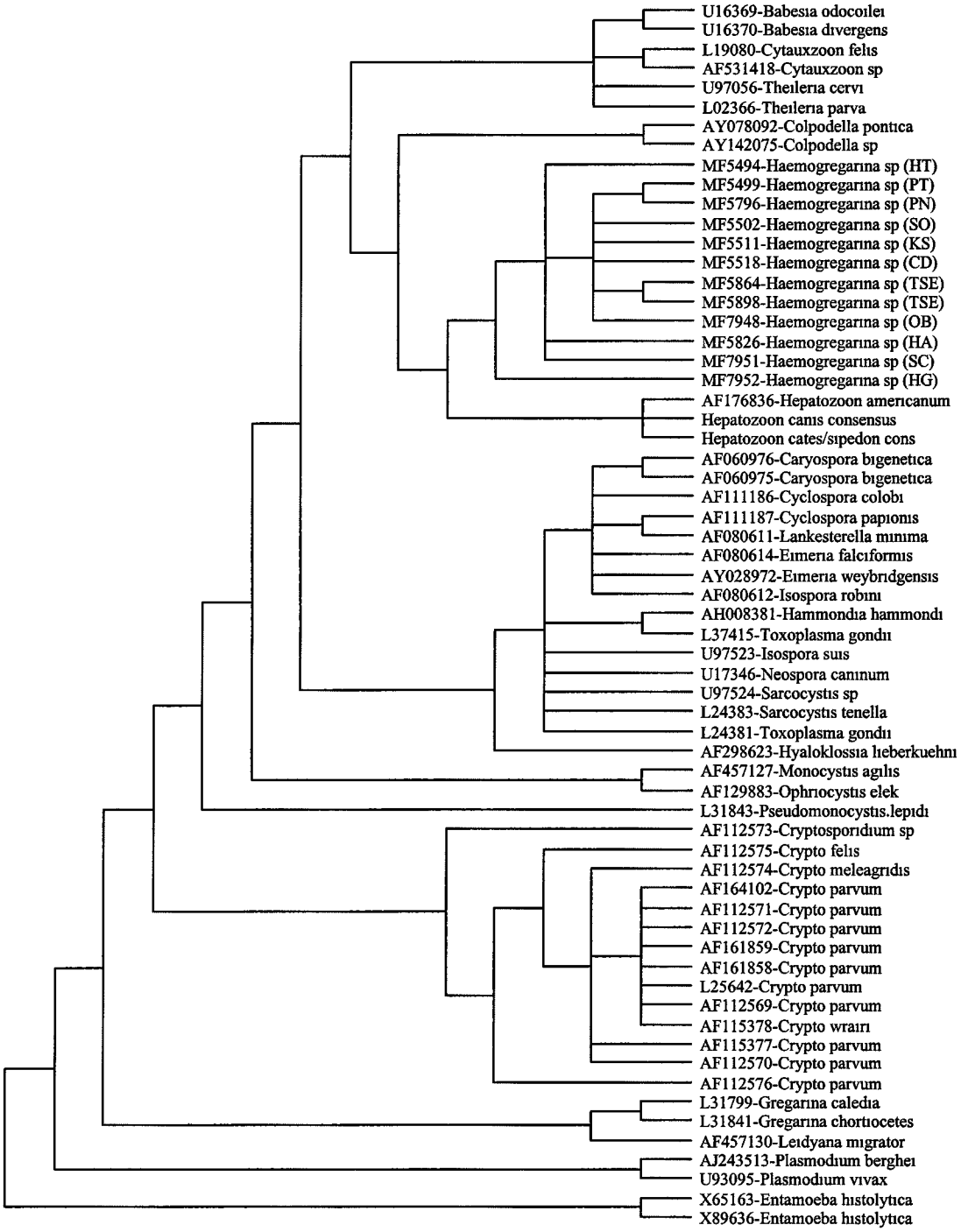


Figure F-17. Dataset B: Strict consensus of 95 most parsimonious trees with gaps treated as 5th base and nucleotides weighted equally. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. The 95 most parsimonious trees had a TL=1004, CI=0.520, RI=0.769, and RC=0.400. The strict consensus collapses into multiple polytomies for most families of Apicomplexa. The order Eugregarinida fails to demonstrate monophyly, *Lankesterella* fails to group with the systematically accepted Eucoccidiorida, *Isospora suis* fails to group within Eimeriidae, and the families Sarcocystidae and Eimeriidae lack resolution. The *Haemogregarina* and *Hepatozoon* genera group with the *Colpodella*, however collapse as polytomies within this clade.

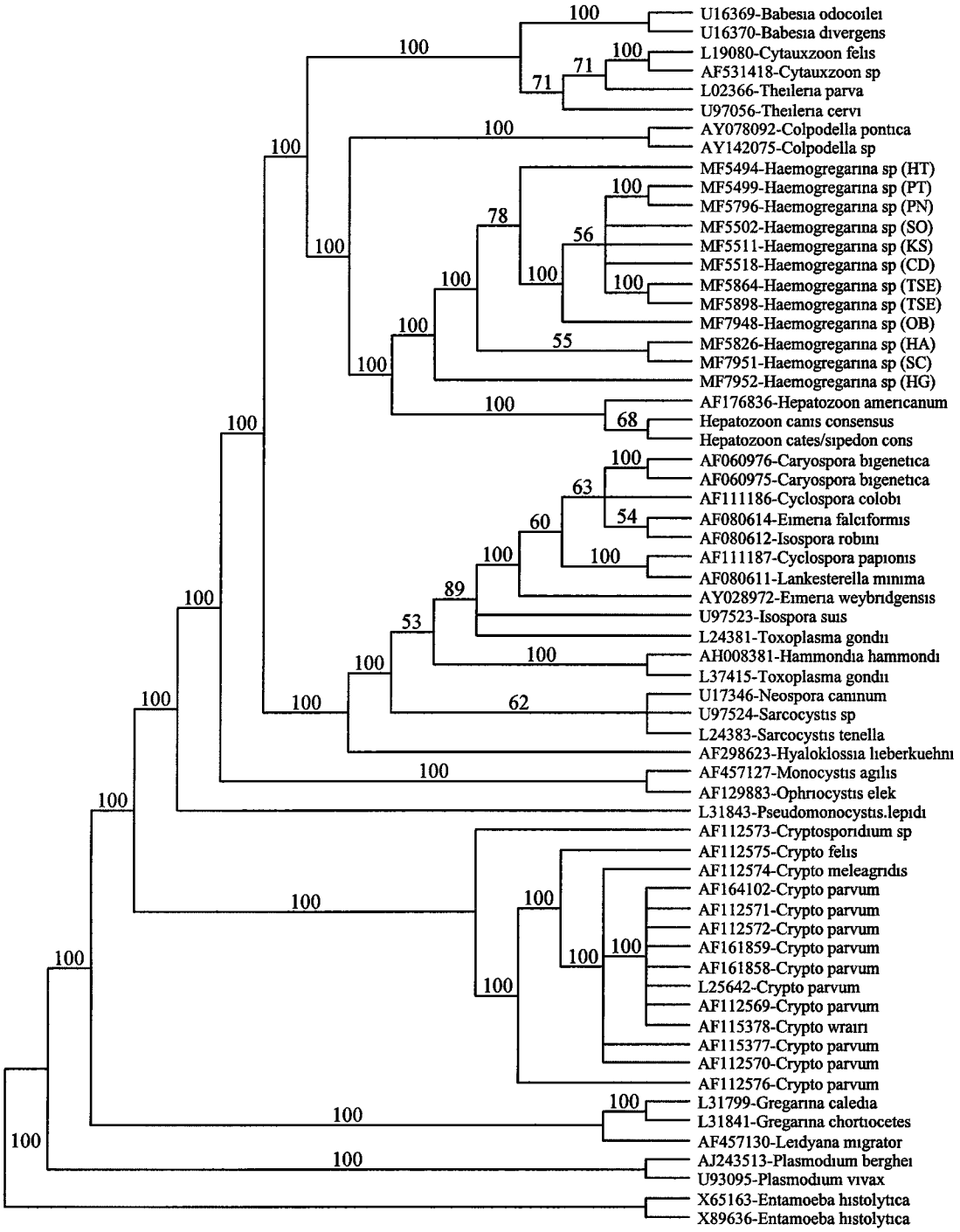


Figure F-18. Dataset B: Majority rule consensus of 95 most parsimonious trees with gaps treated as 5th base. Majority rule values are represented above each branch length. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides weighted equally. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. The 95 most parsimonious trees had a TL=1004, CI=0.520, RI=0.769, and RC=0.400. The majority rule consensus shows that the 95 most parsimonious trees differ in the placement of taxa within Theileriidae, within Haemogregarinidae, and within Eugregarinida.

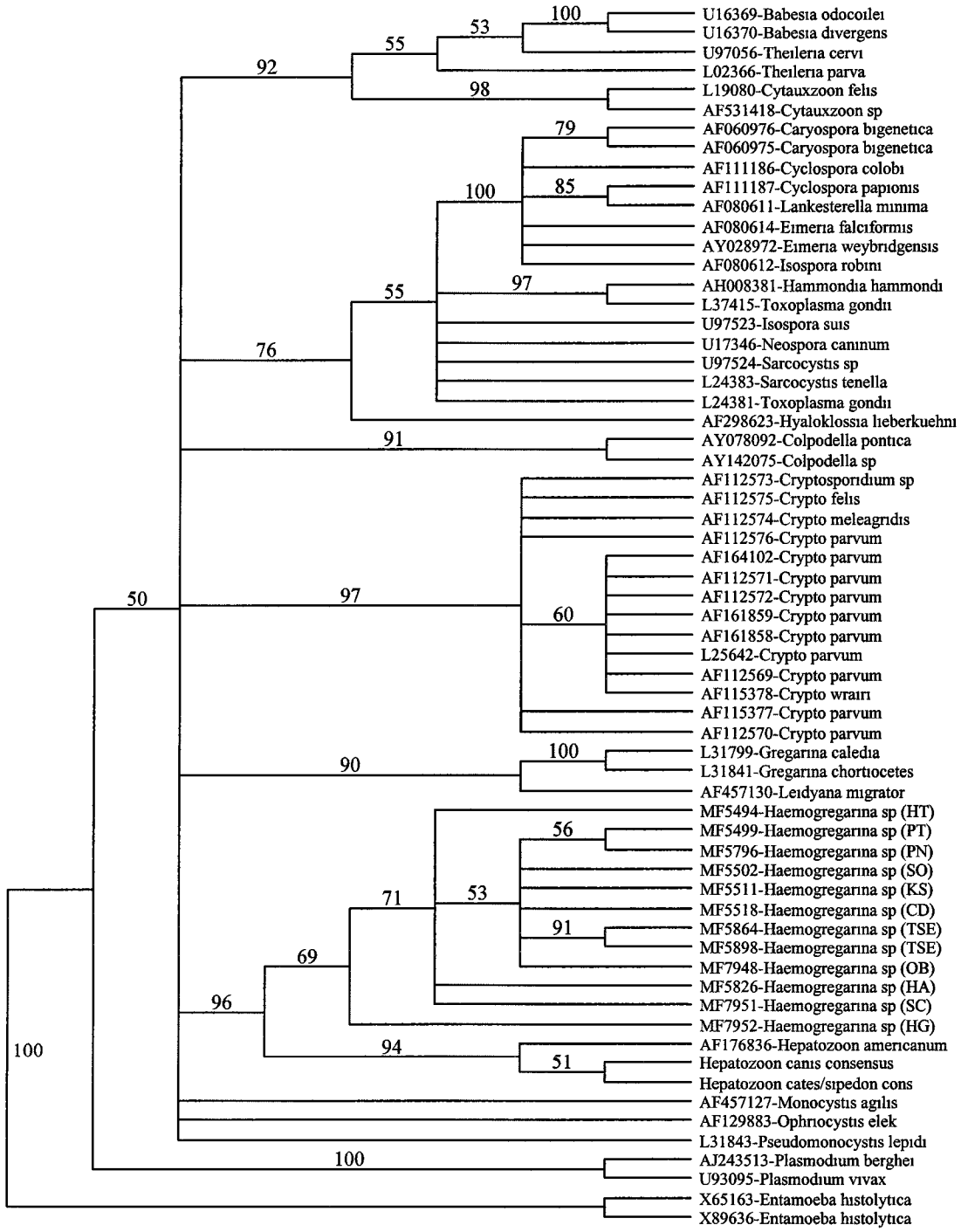


Figure F-19. Dataset B: Bootstrap consensus tree by maximum parsimony with bootstrap values <50% support not shown Bootstrap values are represented above each branch length *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum parsimony, a bootstrap heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. Gaps were treated as 5th base and nucleotides weighted equally The bootstrap consensus topology shows that the overall topology lacks support (bootstrap value = 50) and all major clades collapse into a polytomy.

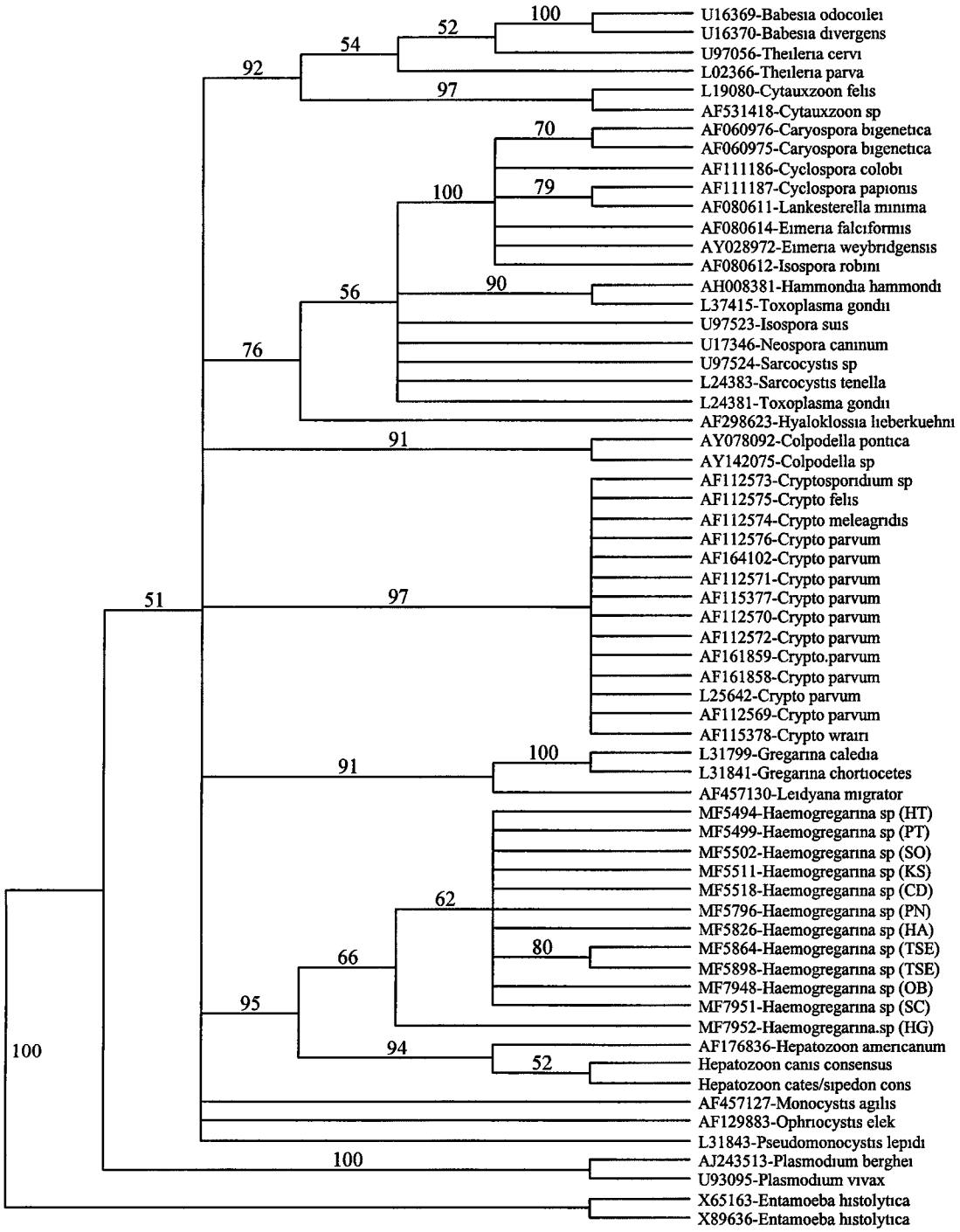


Figure F-20. Dataset B: Jackknife consensus tree by maximum parsimony with jackknife values <50% support not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum parsimony, a jackknife heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. Gaps were treated as 5th base and nucleotides weighted equally. The jackknife consensus topology shows that the overall topology lacks support (jackknife value = 51) and all major clades collapse into a polytomy.

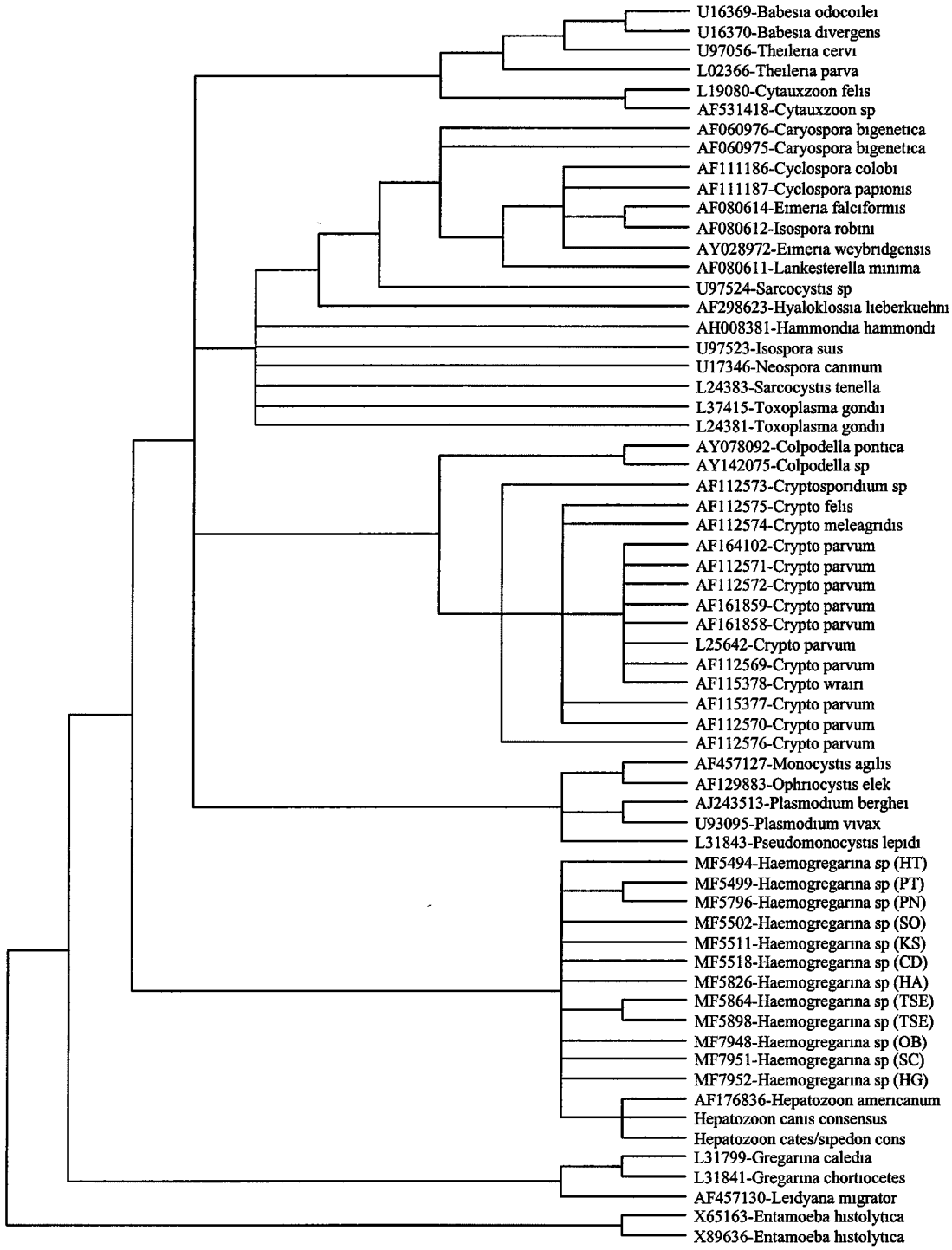


Figure F-21. Dataset B: Strict consensus of 79 most parsimonious trees with gaps treated as missing data. *Entamoeba histolytica* was set as outgroup to the Apicomplexa nucleotides were weighted equally. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. The 79 most parsimonious trees had a TL=695, CI=0.557, RI=0.787, and RC=0.438. The strict consensus almost completely collapses the Sarcocystidae, combines Colpodellidae monophyletic with Cryptosporidiidae, and bifurcates the Eugregarinida. The placement of *Lankesterella*, and *Isospora suis*, show the same systematic problems as seen in all previous topologies.

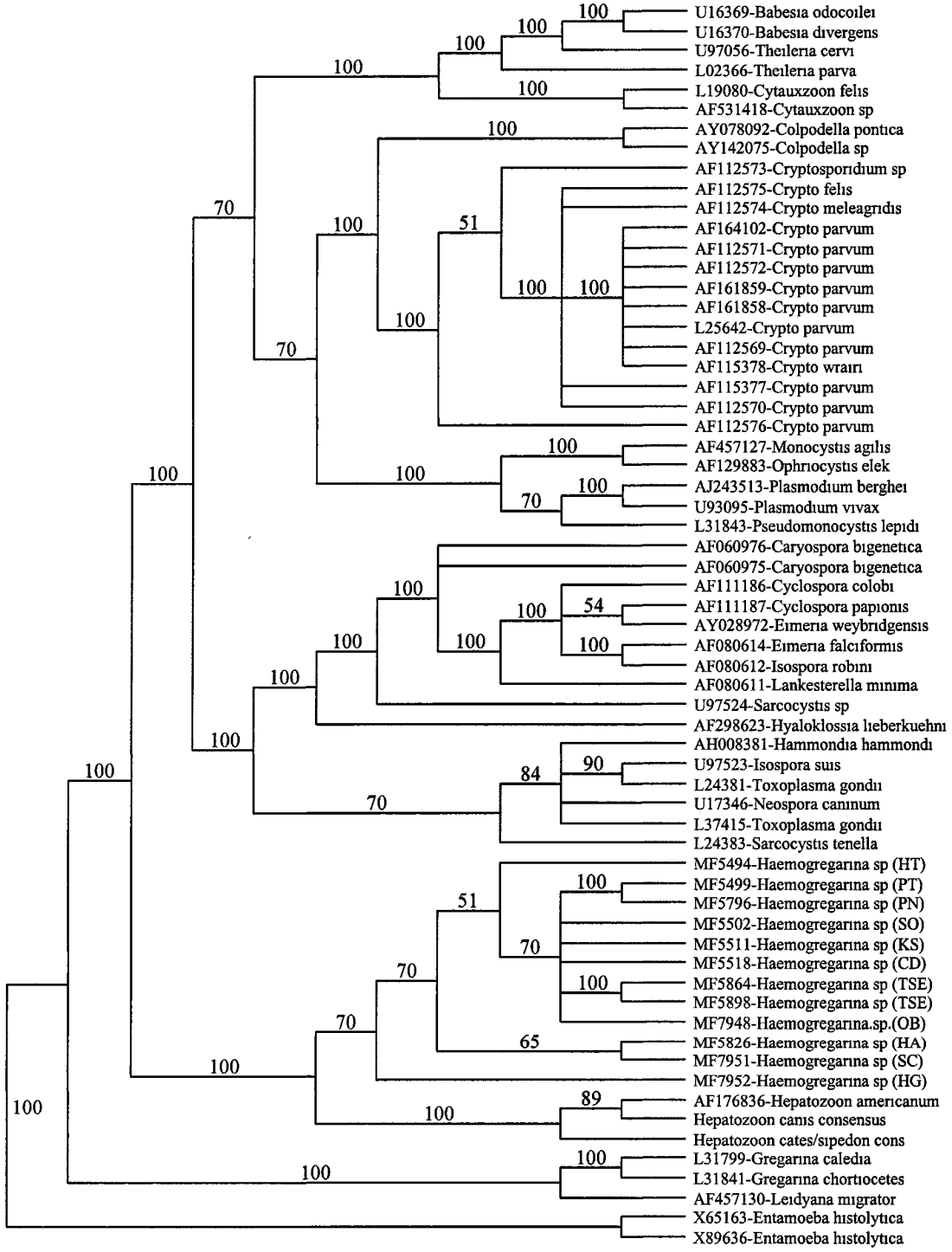


Figure F-22. Dataset B: Majority rule consensus of 79 most parsimonious trees with gaps treated as missing. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides weighted equally. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. The 79 most parsimonious trees had a TL=695, CI=0.557, RI=0.787, and RC=0.438. Majority rule consensus values are represented above each branch. This cladogram bifurcates the Eucoccidiorida families, bifurcates the Gregarina class, and bifurcates genera such as *Sarcocystis* and *Toxoplasma*.

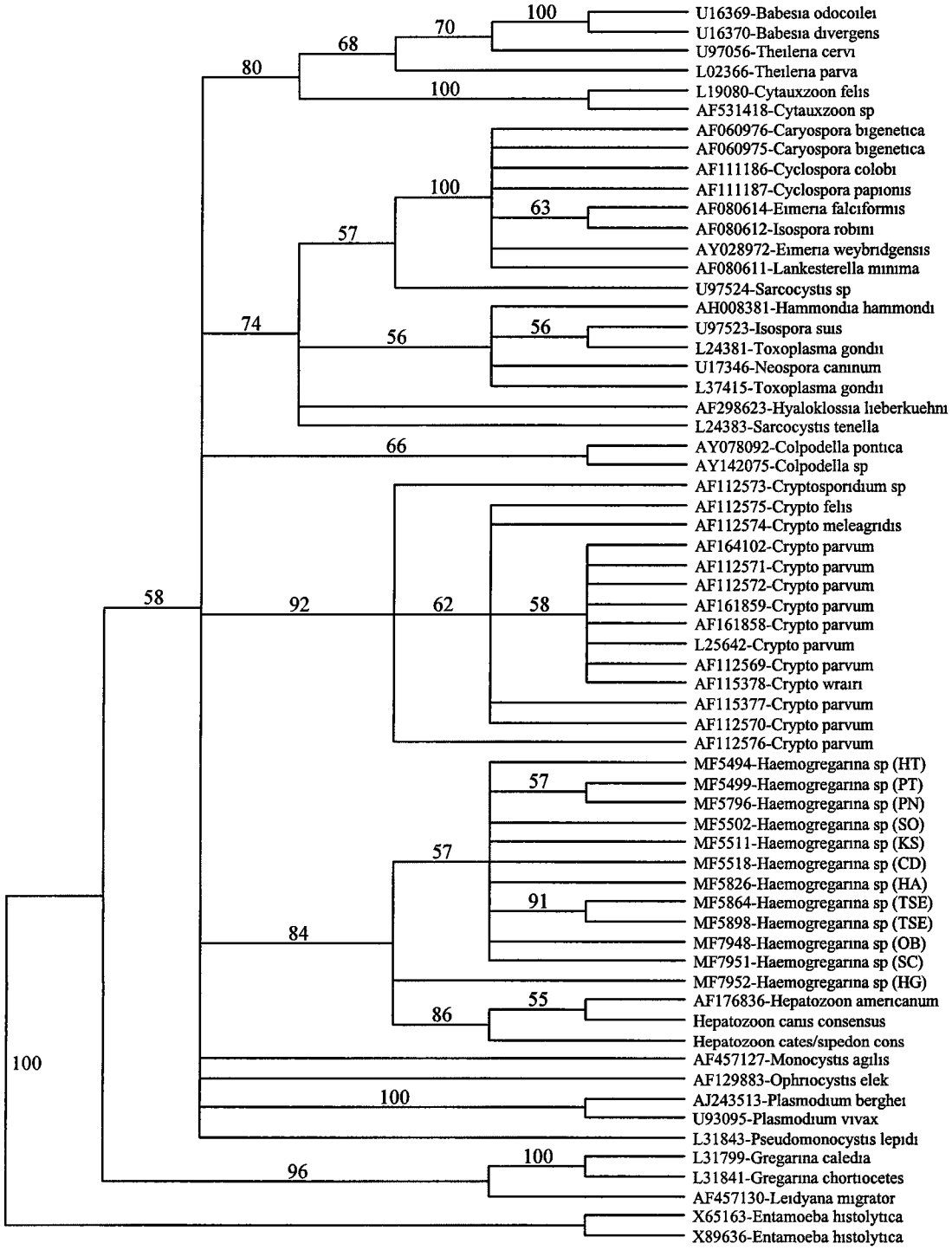


Figure F-23. Dataset B: Bootstrap consensus tree by maximum parsimony with gaps were treated as missing with bootstrap values <50% support not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides weighted equally. With optimality criteria set to maximum parsimony, a bootstrap heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. The topology has weak support for the first major bifurcation (bootstrap = 58) and most of the other clades collapse as polytomies.

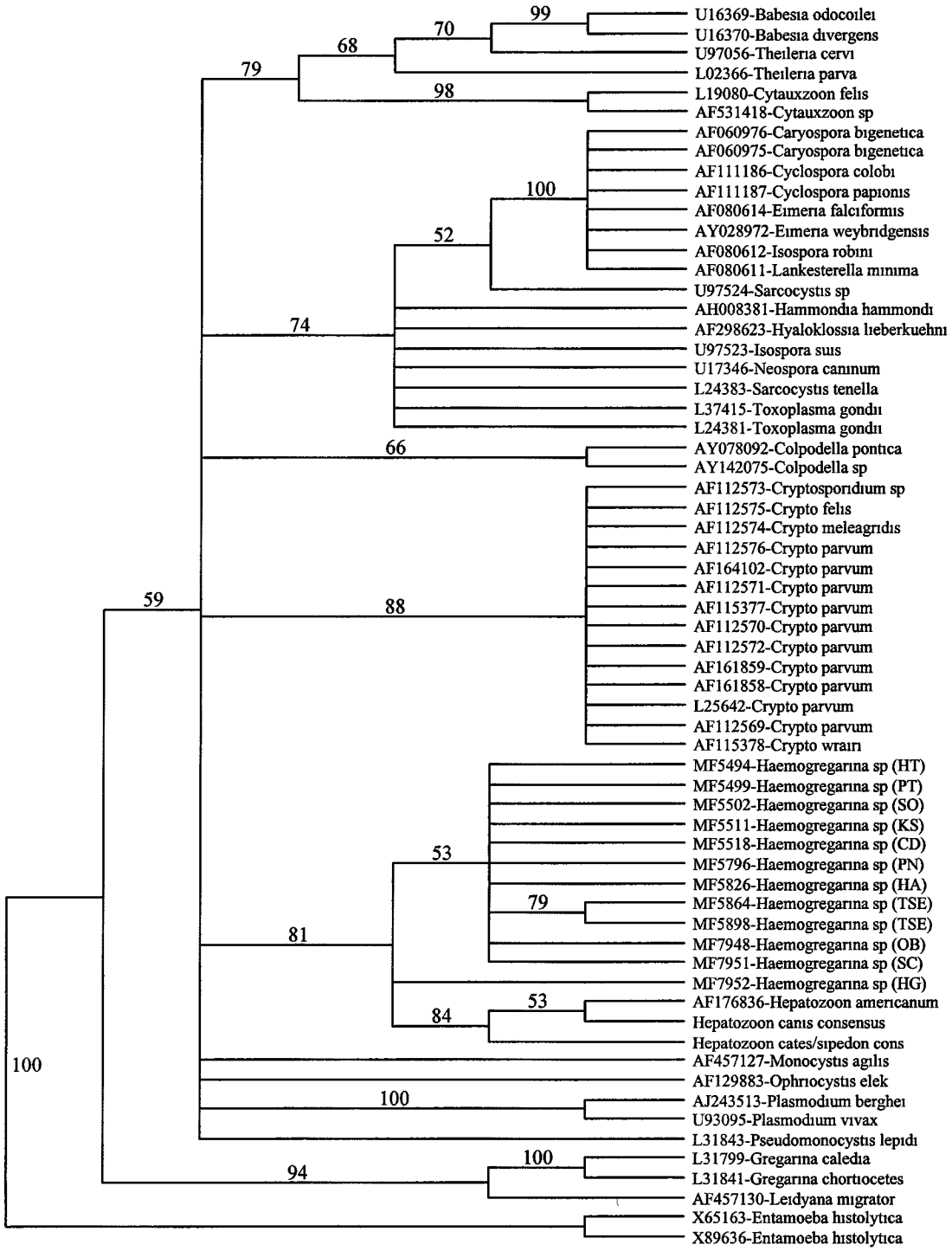


Figure F-24. Dataset B: Jackknife consensus tree by maximum parsimony with gaps were treated as missing with jackknife values <50% support not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides weighted equally. With optimality criteria set to maximum parsimony, a jackknife heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. The topology has weak support for the first major bifurcation (bootstrap = 59) and most of the other clades collapse as polytomies.

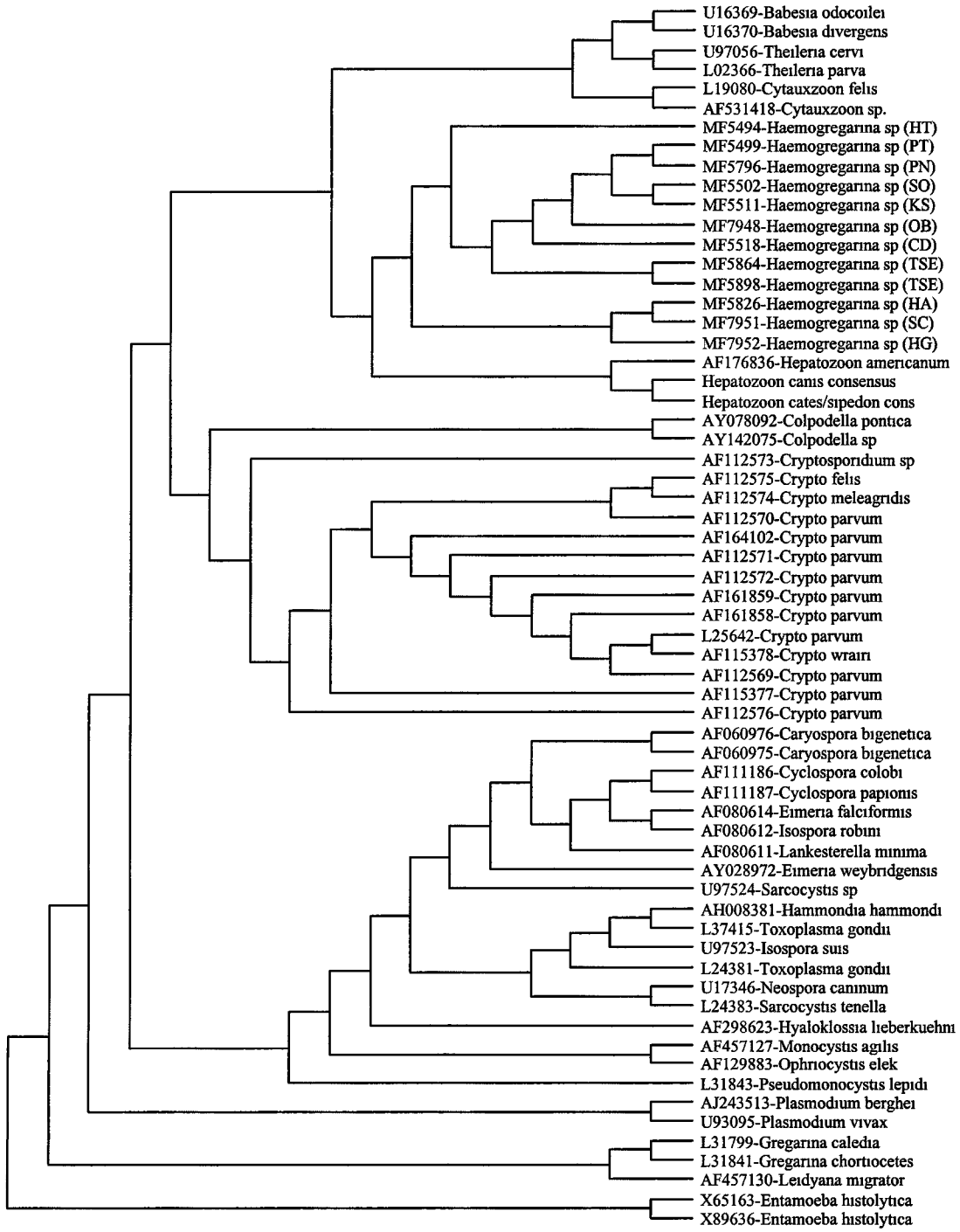


Figure F-25. Dataset B: Cladogram with optimality criterion set to distance with a neighbor joining search and Tajima Nei distance correction algorithm. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally. A neighbor joining search was performed for 1000 replicates by random stepwise addition with TBR branch swap method. The ME value is 1.39749. This topology clusters Eucoccidiorida with Piroplasmida as a monophyletic unit. Sarcocystidae and Eimeriidae form a monophyletic clade, however the Gregarina class bifurcates among them. *Lankesterella* and *Isospora suis* are not grouped with their systematically approved classes. This cladogram bifurcates the Eucoccidiorida families, bifurcates the Gregarina class, and bifurcates genera such as *Sarcocystis* and *Toxoplasma.*

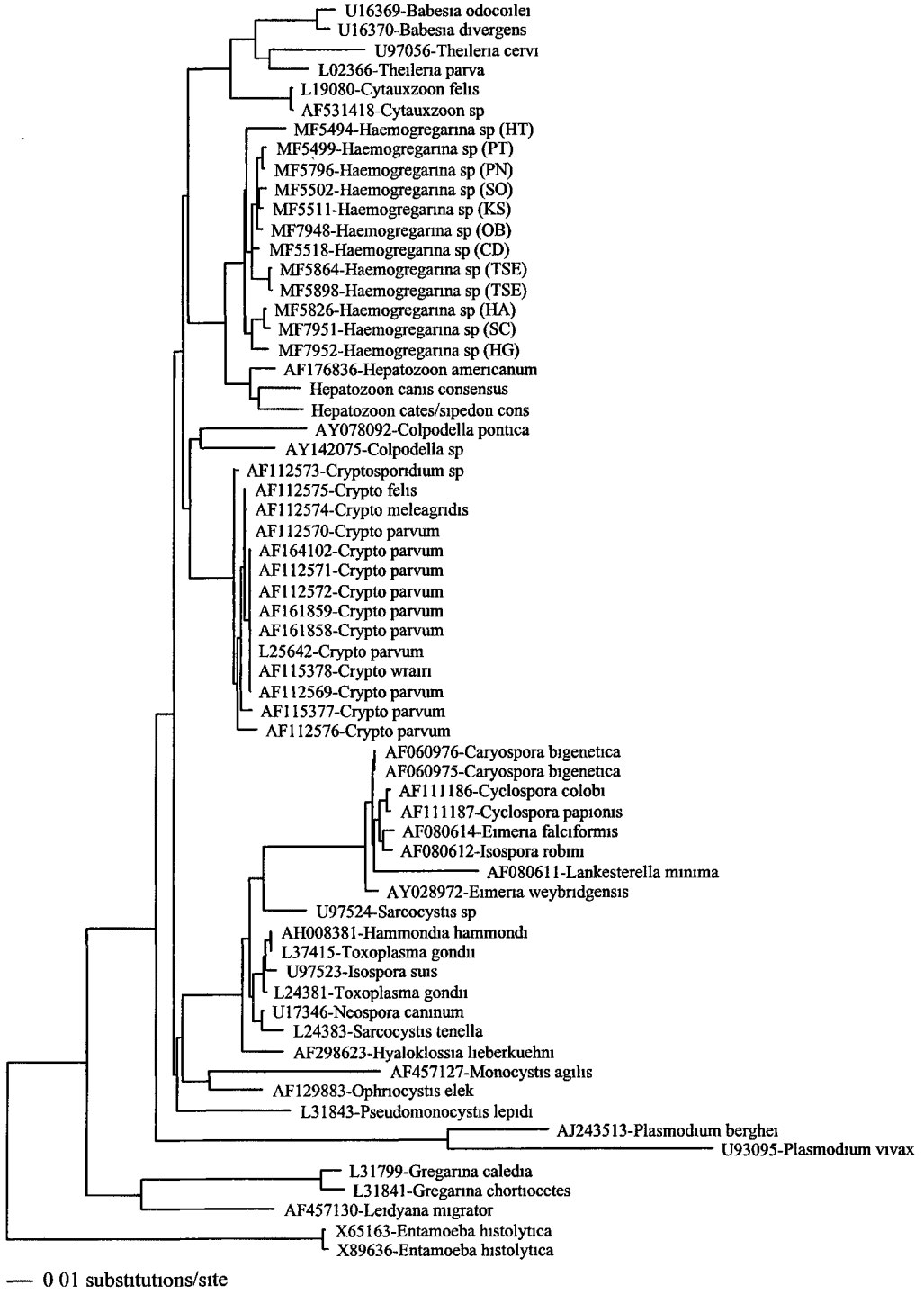


Figure F-26. Dataset B: Phylogram with optimality criterion set to distance with a neighbor joining search and Tajima Nei distance correction algorithm. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally. A neighbor joining search was performed for 1000 replicates by random stepwise addition with TBR branch swap method. The ME value is 1.39749. This topology is identical to that in Figure F-25, except the number of substitutions per site is represented as a phylogram.

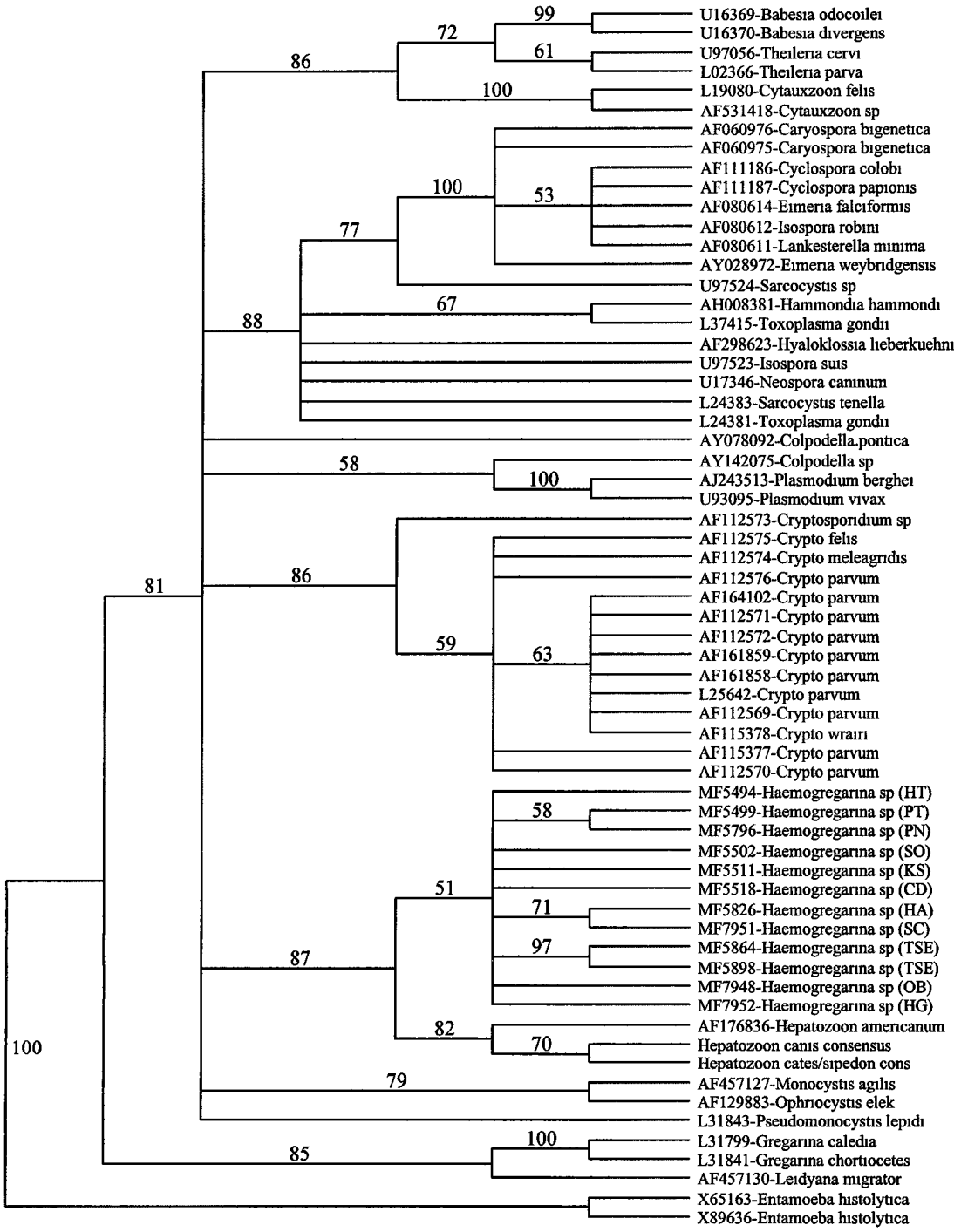


Figure F-27. Dataset B: Bootstrap consensus tree by neighbor joining with bootstrap values <50% support not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally. With optimality criteria set to distance, a bootstrap neighbor-joining search was performed for 2500 replicates. This topology shows relatively strong support the first bifurcation, however the majority of the clades collapse as polytomies. The Haemogregarinidae family is strongly supported and show monophyletic clades of *Haemogregarina* and *Hepatozoon* respectively. The placement of *Plasmodium* outside the Gregarina is weakly supported. *Toxoplasma* and *Sarcocystis* fail to group identical genera monophyletically.

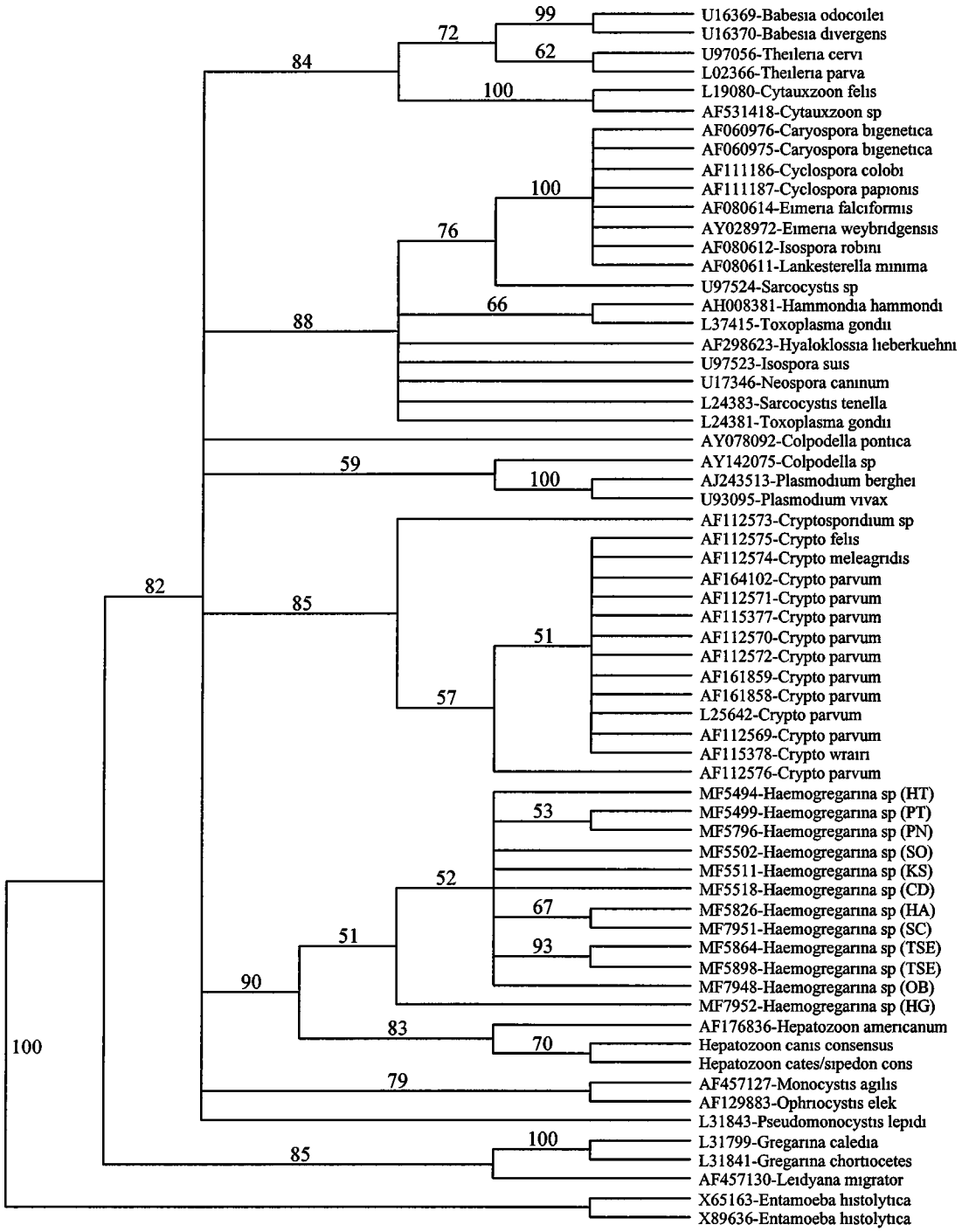


Figure F-28. Dataset B: Jackknife consensus tree by maximum parsimony with jackknife values <50% support not shown. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally. With optimality criteria set to distance, a jackknife neighbor-joining search was performed for 2500 replicates. This topology shows relatively strong support the first bifurcation, however the majority of the clades collapse as polytomies. The Haemogregarinidae family is strongly supported and show monophyletic clades of *Haemogregarina* and *Hepatozoon* respectively. The placement of *Plasmodium* outside the Gregarina is weakly supported. *Toxoplasma* and *Sarcocystis* fail to group identical genera monophyletically.

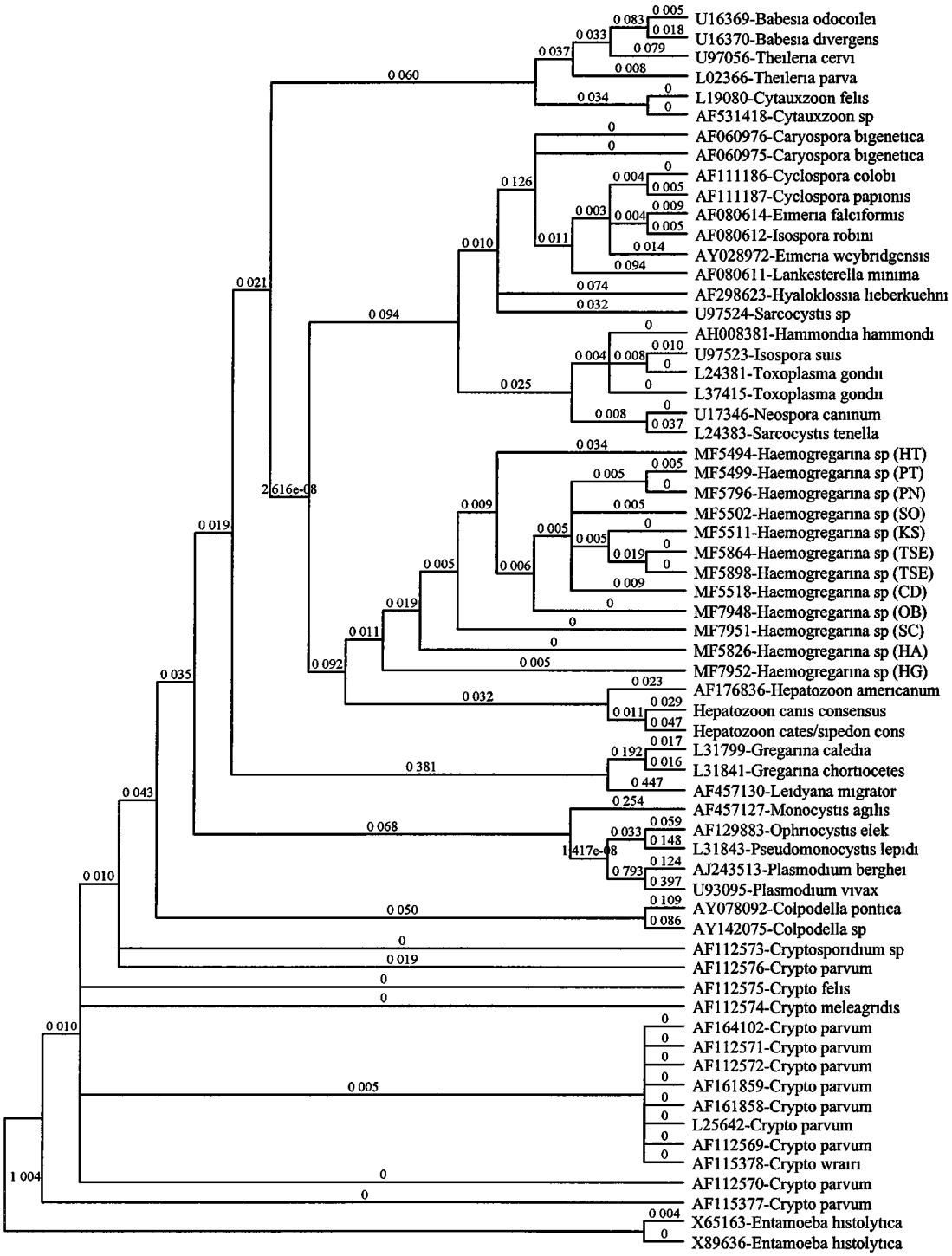


Figure F-29. Dataset B: Maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model TrN + G; base frequencies of A=0.3151, C=0.1648, G=0.2307, T=0.2894; substitution rate matrix of A-C=1.0000, A-G=1.9070, A-T=1.0000, C-G=1.0000, C-T=4.0781, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2339. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally. With optimality criteria set to maximum likelihood, a heuristic search was performed with TBR branch swap method and ran until completion. The –lnL score is 3774.12108.

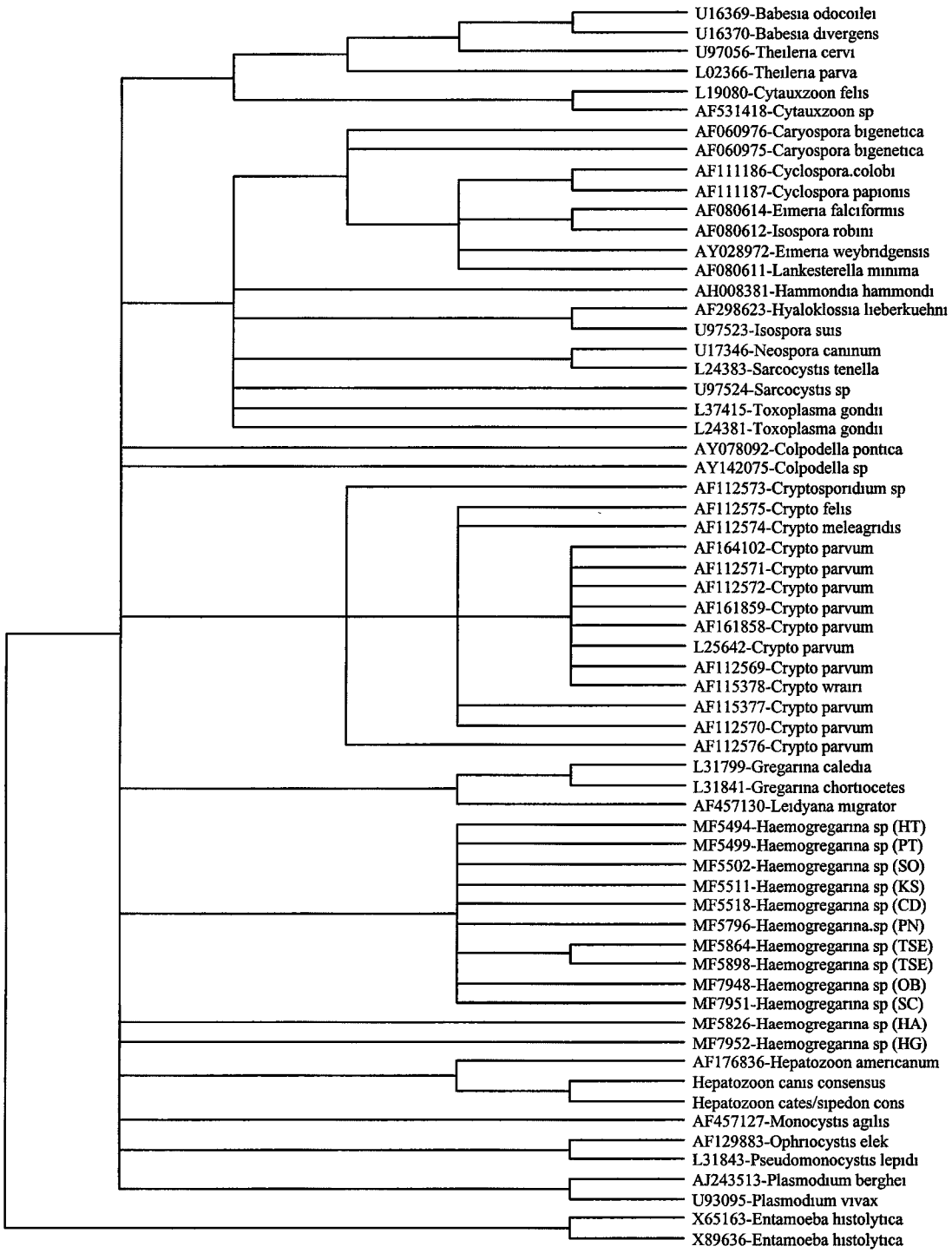


Figure F-30. Dataset B: Bootstrap consensus with maximum likelihood using Modeltest parameters. The parameters included model TrN + G; base frequencies of A=0.3151, C=0.1648, G=0.2307, T=0.2894; substitution rate matrix of A-C=1.0000, A-G=1.9070, A-T=1.0000, C-G=1.0000, C-T=4.0781, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2339. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum likelihood, a bootstrap heuristic search was performed for 2500 replicates by neighbor joining with TBR branch swap method. Bootstrap values with <50% support are not shown.

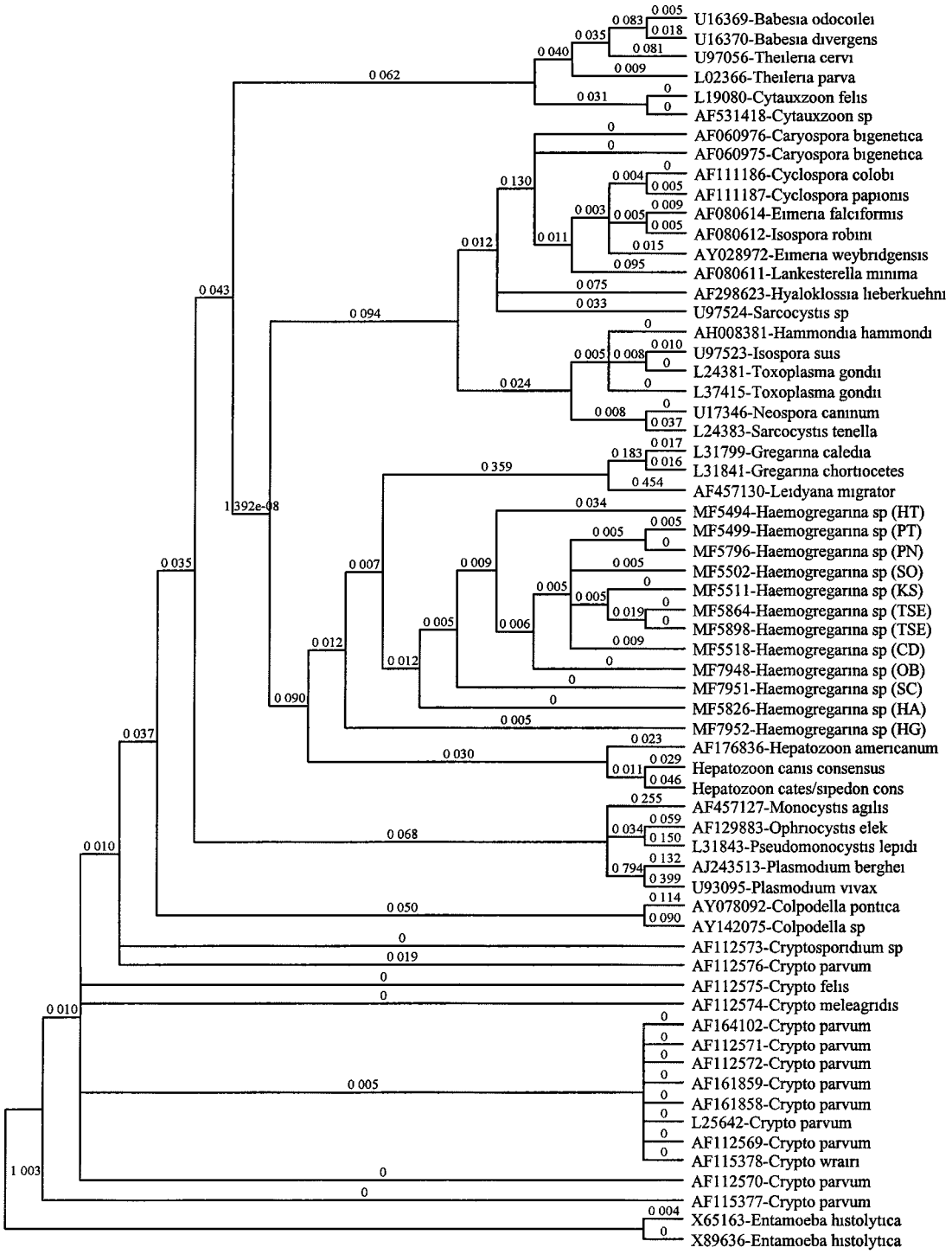


Figure F-31. Dataset B· Maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters. The parameters included model TrN + G; base frequencies of A=0.2937, C=0.1655, G=0.2484, T=0.2924; substitution rate matrix of A-C=1.6907, A-G=2.4927, A-T=1.5261, C-G=0.8853, C-T=5.1433, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2338. *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally. With optimality criteria set to maximum likelihood, a heuristic search was performed with TBR branch swap method and ran until completion. The –ln L score is 3770.59511. This topology is almost identical to Figure F-29 (different Modeltest parameters) yet differs in topology in the placement of the *Gregarina-Leidyana* monophyletic clade.

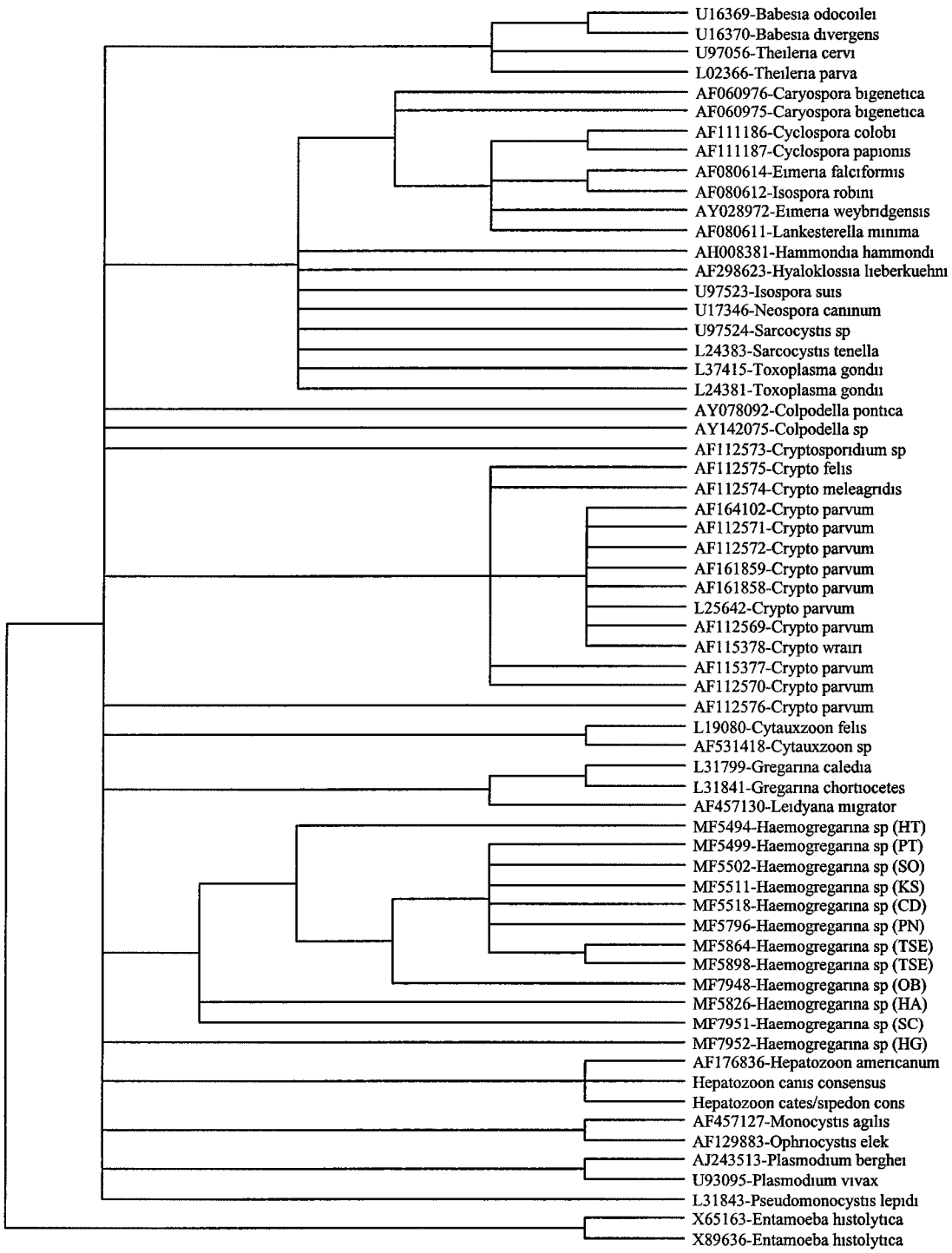


Figure F-32. Dataset B: Bootstrap consensus for maximum likelihood using Modeltest parameters. The parameters included model TrN + G; base frequencies of A=0.2937, C=0.1655, G=0.2484, T=0.2924; substitution rate matrix of A-C=1.6907, A-G=2.4927, A-T=1.5261, C-G=0.8853, C-T=5.1433, and G-T=1.0000; ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2338. *Entamoeba histolytica* was set as outgroup to the Apicomplexa. With optimality criteria set to maximum likelihood, a bootstrap heuristic search was performed for 2500 replicates by neighbor joining with TBR branch swap method. Bootstrap values with <50% support are not shown.

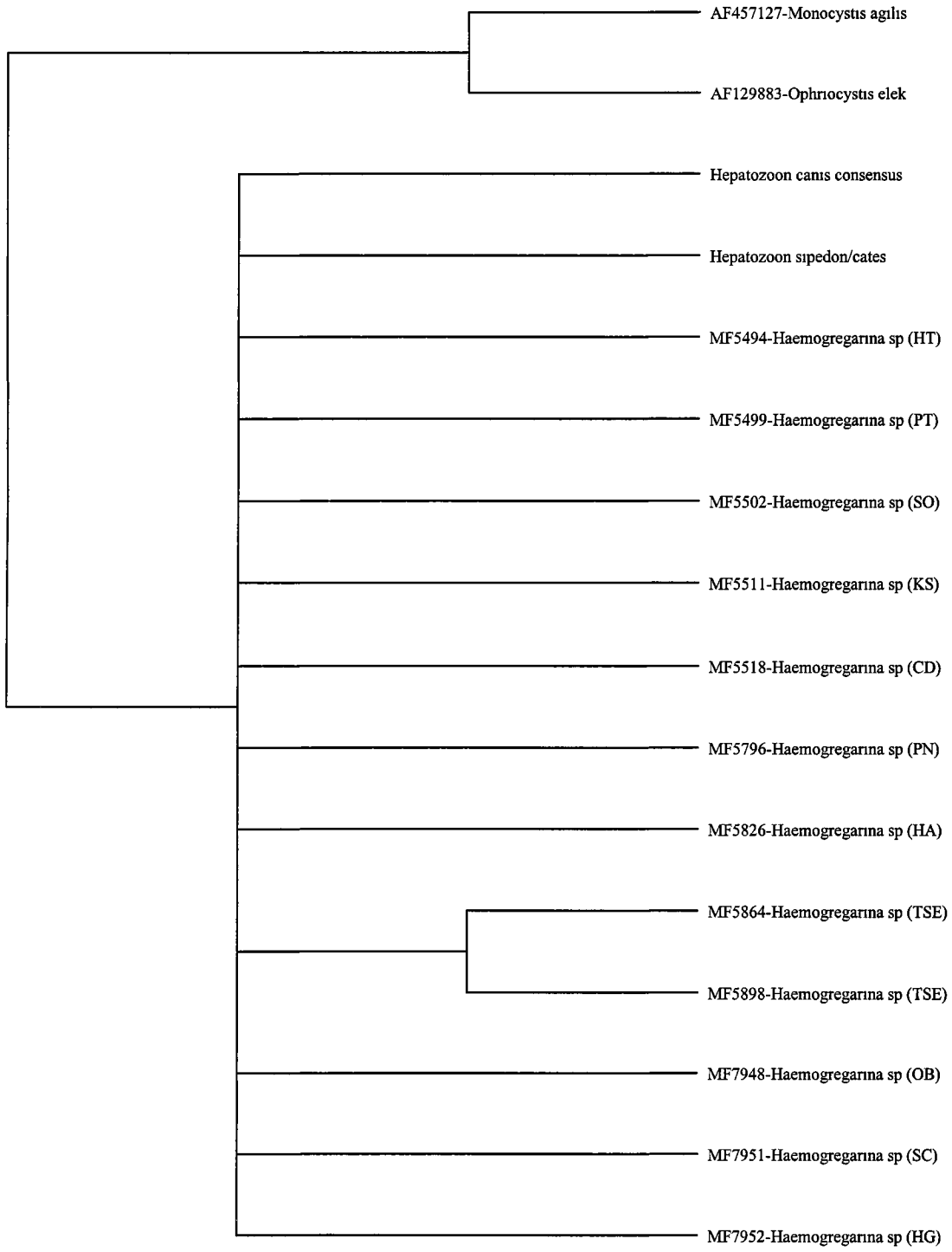


Figure F-33 Dataset C Strict consensus of 55 most parsimonious trees. *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. Gaps were treated as 5th base and nucleotides weighted equally The 55 most parsimonious trees had a TL=136, CI=0.882, RI=0.800, and RC=0 706. All clades collapse as a polytomy with the exception to the TSE hosts *Haemogregarina* sp. hosts are abbreviated as. HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonorianse*, TSE=*Trachemys scripta elegans*

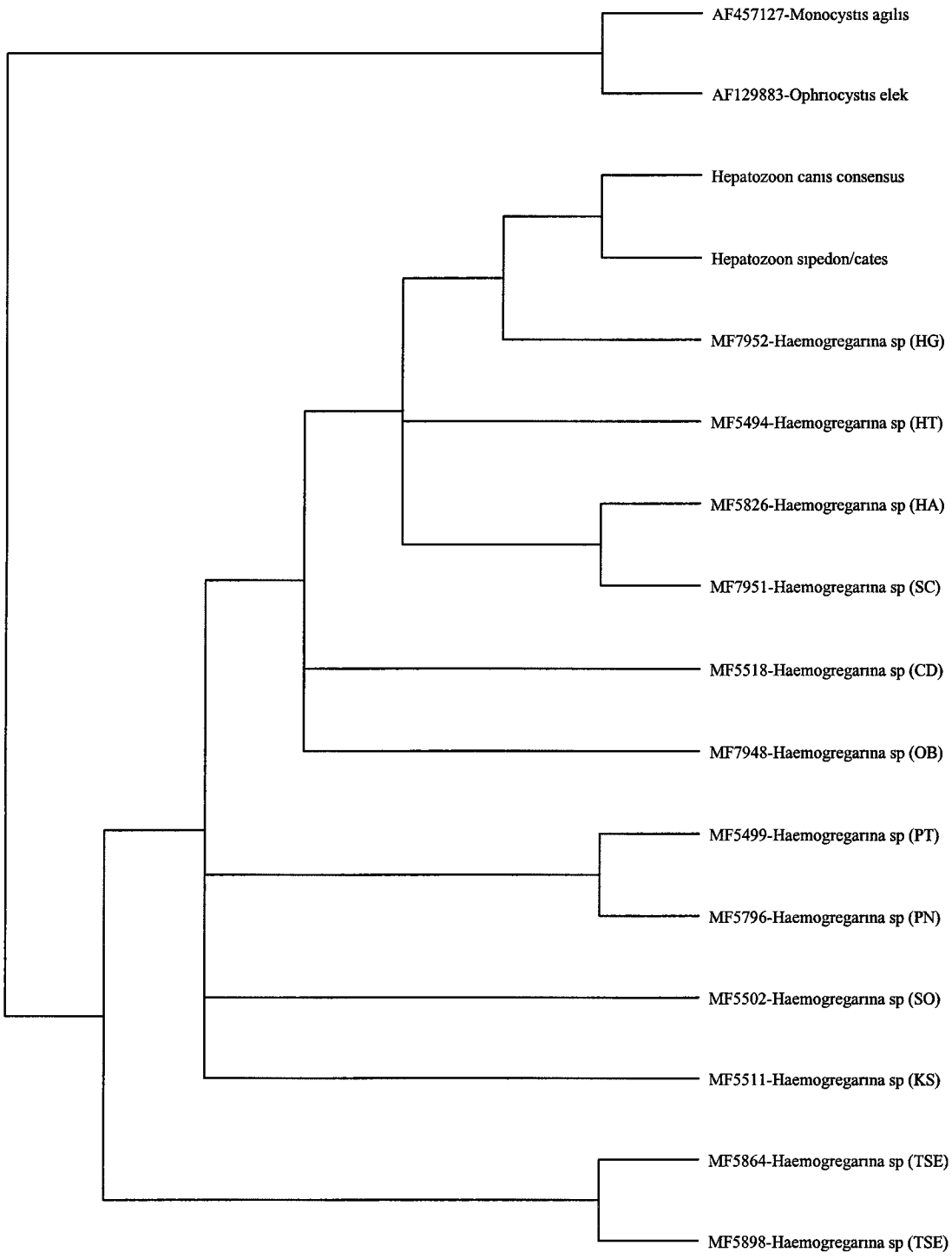


Figure F-34 Dataset C Majority rule consensus of 55 most parsimonious trees with majority rule values represented above each branch *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method Gaps were treated as 5th base and nucleotides weighted equally The 55 most parsimonious trees had a TL=136, CI=0 882, RI=0 800, and RC=0 706 This toplogy collapses into a polytomy with the exception to the TSE host clade, the HA-SC clade, and the PT-PN clade *Hepatozoon* falls monophyletic within the *Haemogregarina* clade, thus not showing genera differentiation *Haemogregarina* sp hosts are abbreviated as HA=*Heteremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonoriense*, TSE=*Trachemys scripta elegans*

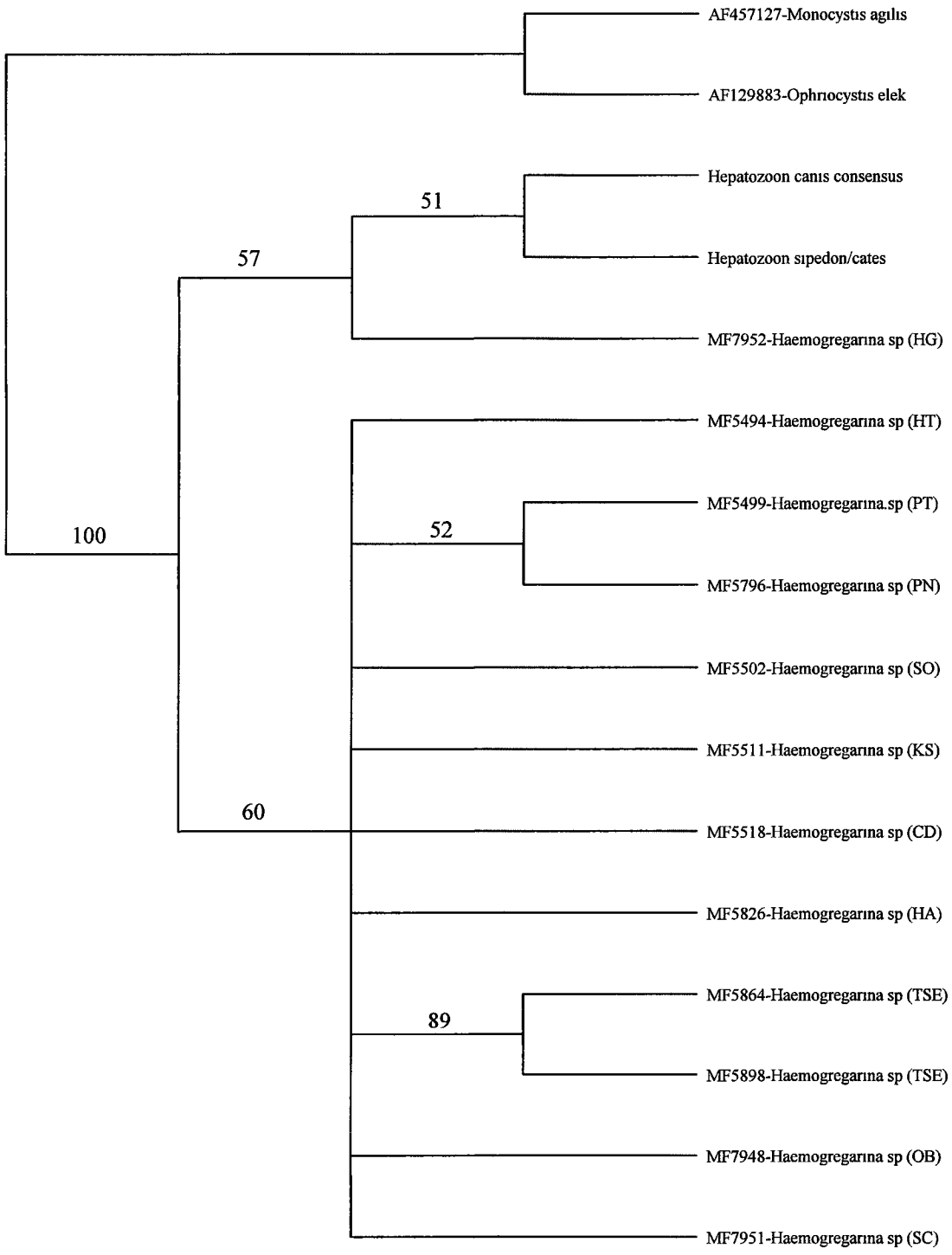


Figure F-35 Dataset C Bootstrap consensus tree by maximum parsimony with bootstrap values <50% support not shown *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae With optimality criteria set to maximum parsimony, a bootstrap heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method Gaps were treated as 5th base and nucleotides weighted equally This topology shows MF7952 *Haemogregarina* sp (HG) is monophyletic with *Hepatozoon*, however this is weakly supported at bootstrap value of 57 The PT-PN clade is also weakly supported within Haemogregarina, however the TSE clade shows strong support *Haemogregarina* sp hosts are abbreviated as HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonoriense*, TSE=*Trachemys scripta elegans*

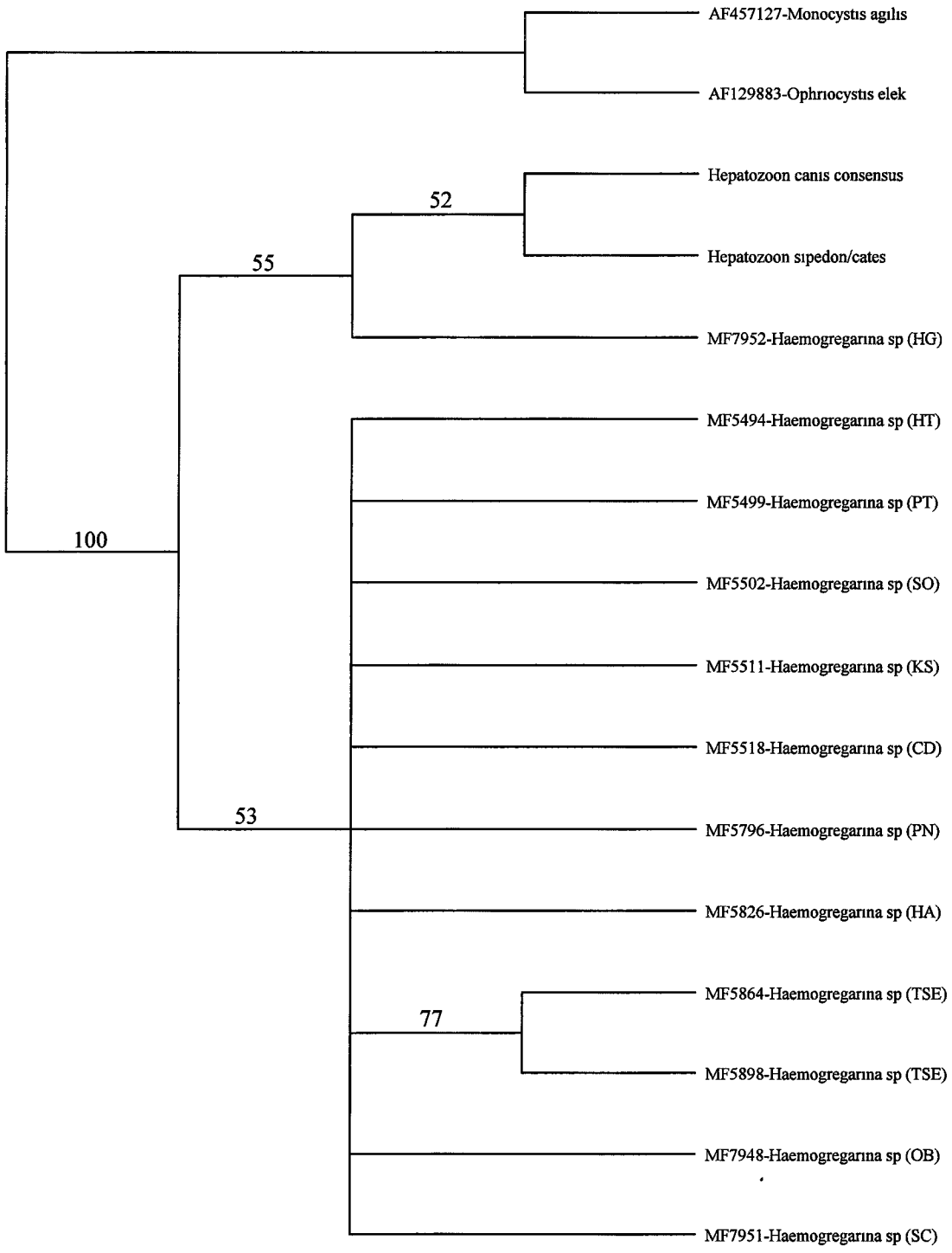


Figure F-36 Dataset C Jackknife consensus tree by maximum parsimony with jackknife values <50% support not shown *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae With optimality criteria set to maximum parsimony, a jackknife heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method Gaps were treated as 5th base and nucleotides weighted equally This topology shows MF7952 *Haemogregarina* sp (HG) is monophyletic with *Hepatozoon*, however this is weakly supported at bootstrap value of 57 The PT-PN clade is not supported (as shown in Figure F-35) within Haemogregarina, however the TSE clade shows strong support *Haemogregarina* sp hosts are abbreviated as HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonoriense*, TSE=*Trachemys scripta elegans*

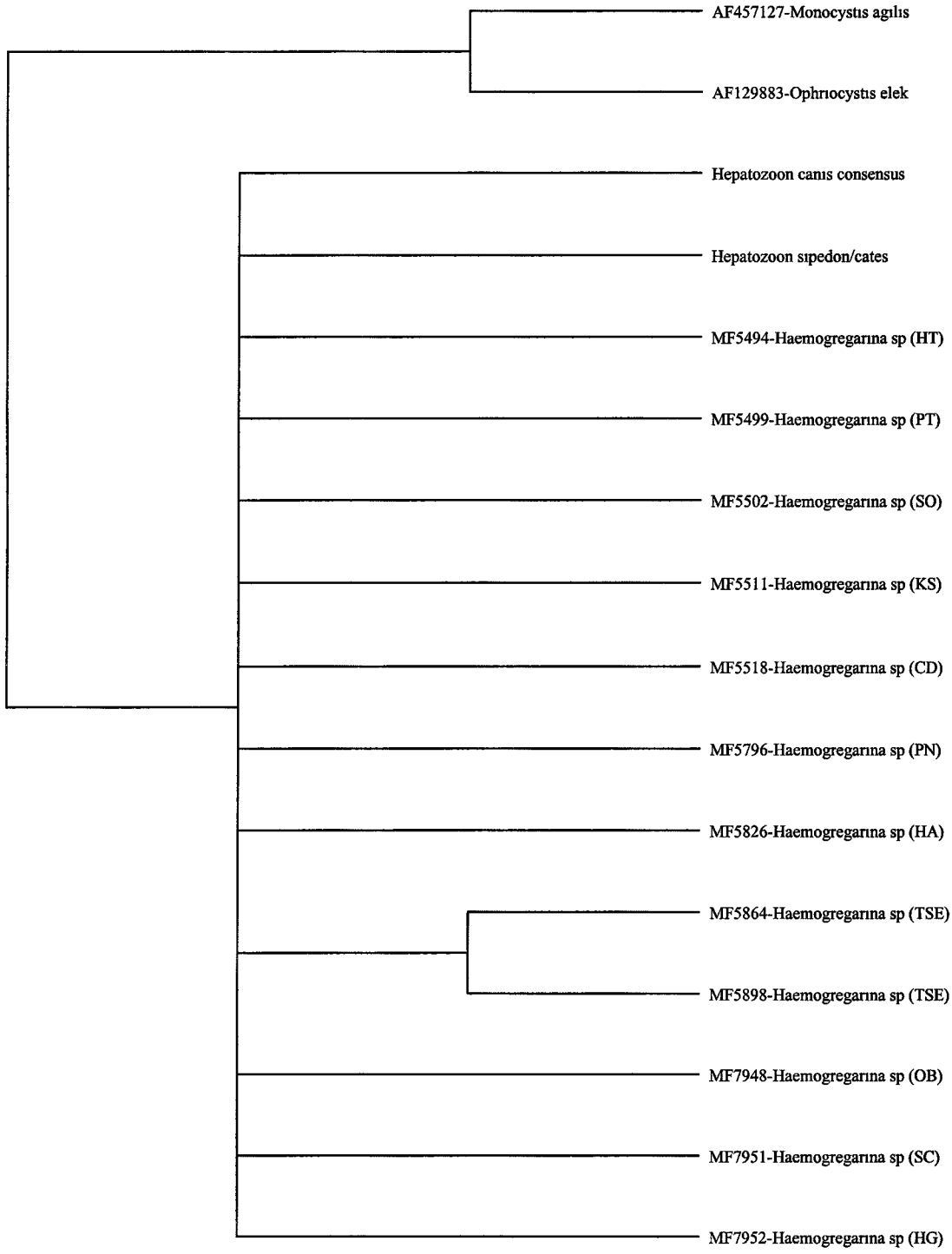


Figure F-37 Dataset C Strict consensus of 81 most parsimonious trees with gaps treated as missing data *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae and nucleotides were weighted equally With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method The 81 most parsimonious trees had a TL=116, CI=0 862, RI=0 787, and RC=0 678. The only distinguishable clade is the TSE-TSE monophyletic unit, and all other taxa form a polytomy *Haemogregarina* sp. hosts are abbreviated as HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonoriense*, TSE=*Trachemys scripta elegans*

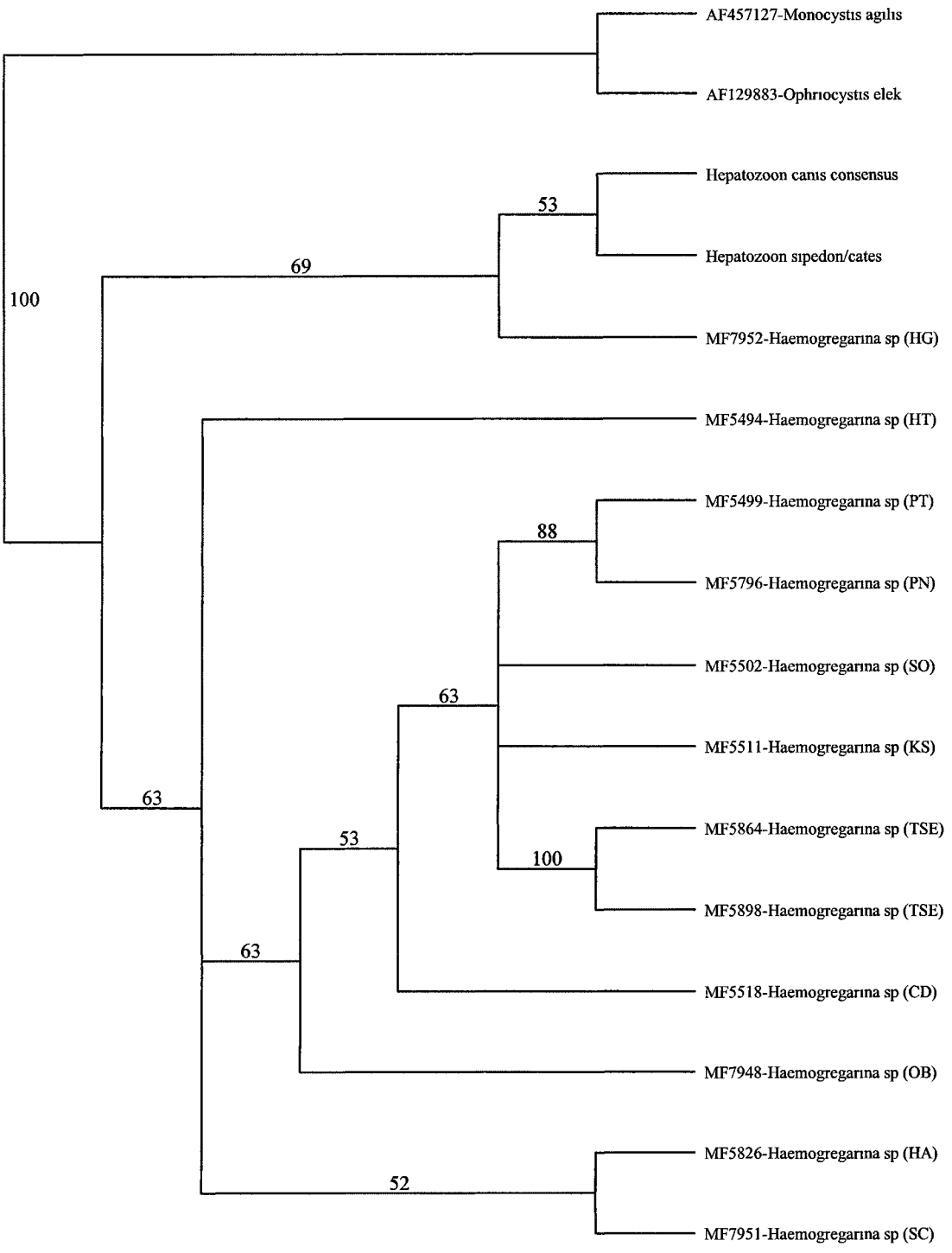


Figure F-38 Dataset C Majority rule consensus of 81 most parsimonious trees with Gaps treated as missing data *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae and nucleotides were weighted equally With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method The 81 most parsimonious trees had a TL=116, CI=0 862, RI=0 787, and RC=0 678 This topology groups *Haemogregarina* sp (HG) as monophyletic with *Hepatozoon* The majority of the trees bifurcate with the TSE-TSE clade and the PT-PN clade *Haemogregarina* sp hosts are abbreviated as HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonoriense*, TSE=*Trachemys scripta elegans*

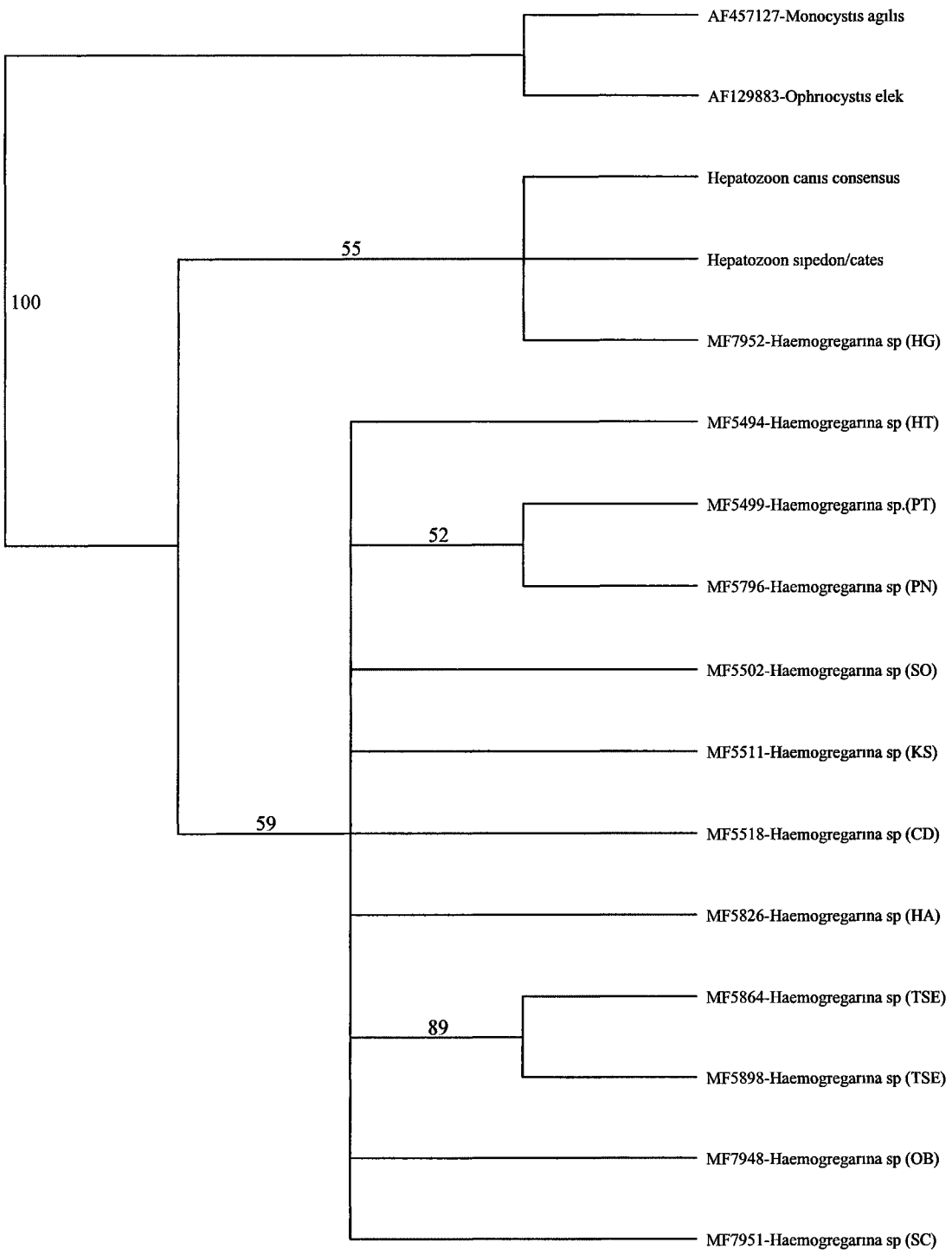


Figure F-39 Dataset C Bootstrap consensus tree by maximum parsimony with bootstrap values <50% support not shown *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae. With optimality criteria set to maximum parsimony, a bootstrap heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method Gaps were treated as missing and nucleotides weighted equally. The only strongly supported clade is the TSE-TSE monophyletic unit *Haemogregarina* sp hosts are abbreviated as HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonorianse*, TSE=*Trachemys scripta elegans*

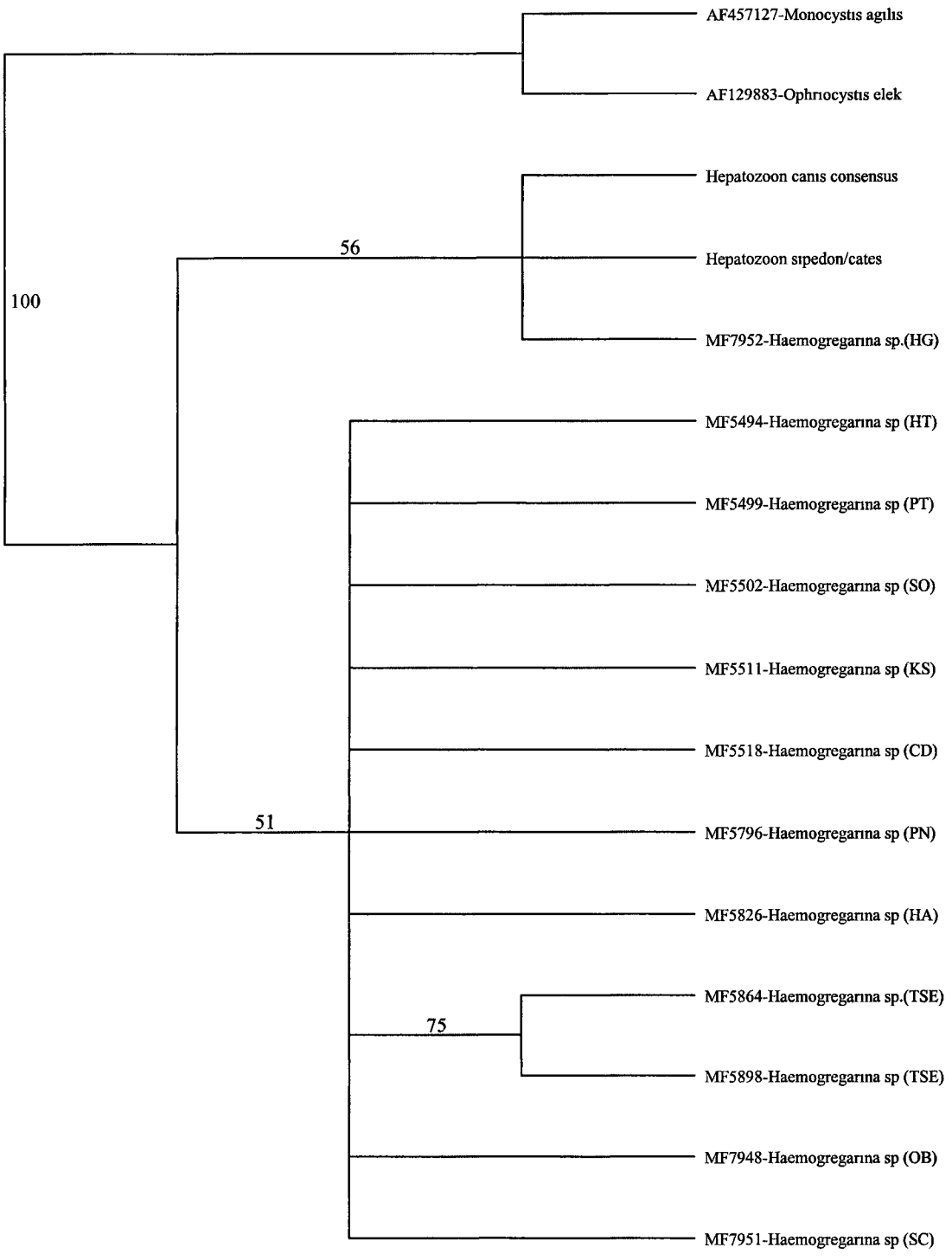


Figure F-40 Dataset C Jackknife consensus tree by maximum parsimony with jackknife values <50% support not shown *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae and nucleotides were weighted equally With optimality criteria set to distance, a jackknife neighbor-joining search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. The only strongly supported clade is the TSE-TSE monophyletic unit *Haemogregarina* sp hosts are abbreviated as HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonorianse*, TSE=*Trachemys scripta elegans*

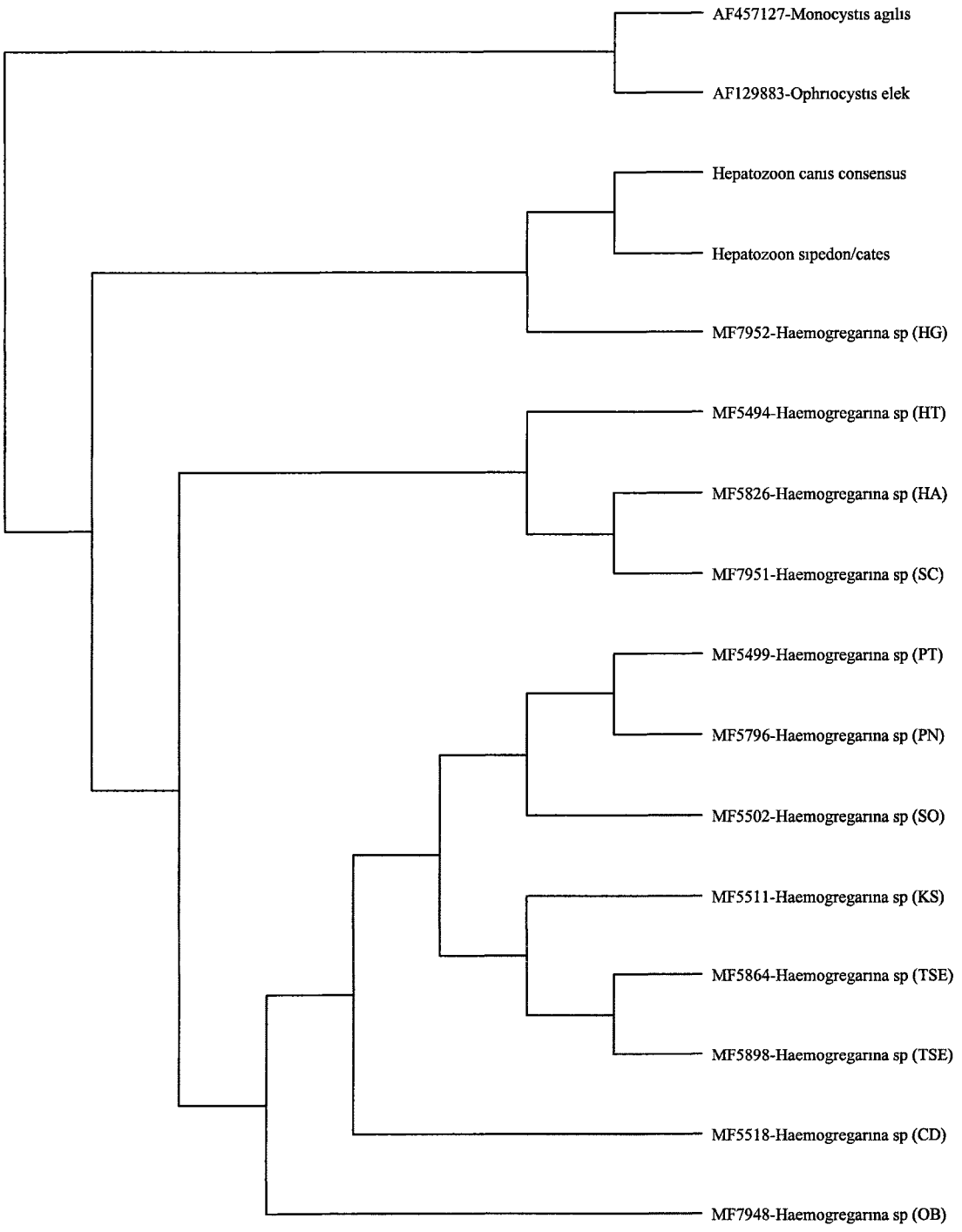


Figure F-41 Dataset C Cladogram with optimality criterion set to distance with a neighbor joining search and Tajima Nei distance correction algorithm *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae and nucleotides were weighted equally A neighbor joining search was performed for 1000 replicates by random stepwise addition with TBR branch swap method The ME value is 0 21982. *Haemogregarina* (HG) forms a monophyletic clade with *Hepatozoon*. HT-HA-SC forms an Old World monophyletic unit, and PT-PN-SO-KS-TSE-TSE clade forms a New World monophyletic unit *Haemogregarina* sp hosts are abbreviated as: HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonorianse*, TSE=*Trachemys scripta elegans*.

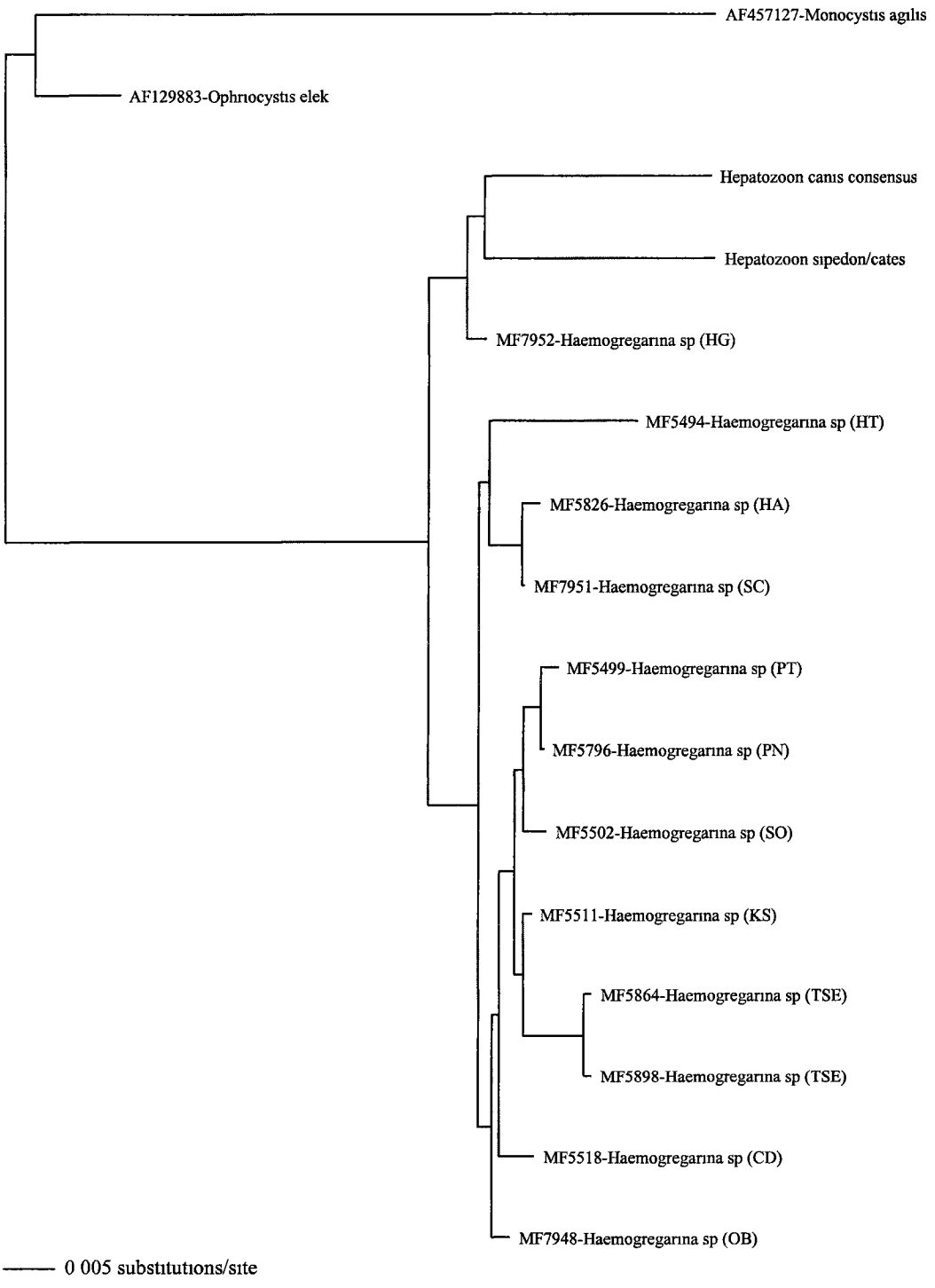


Figure F-42 Dataset C Phylogram with optimality criterion set to distance with a neighbor joining search and Tajima Nei distance correction algorithm. *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae and nucleotides were weighted equally A neighbor joining search was performed for 1000 replicates by random stepwise addition with TBR branch swap method. The number of substitutions per site is represented as the length of each branch and the ME score is 1.39749 for the phylogram This topology is identical to Figure 41 however it is represented as a phylogram *Haemogregarina* sp. hosts are abbreviated as: HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonoriense*, TSE=*Trachemys scripta elegans*.

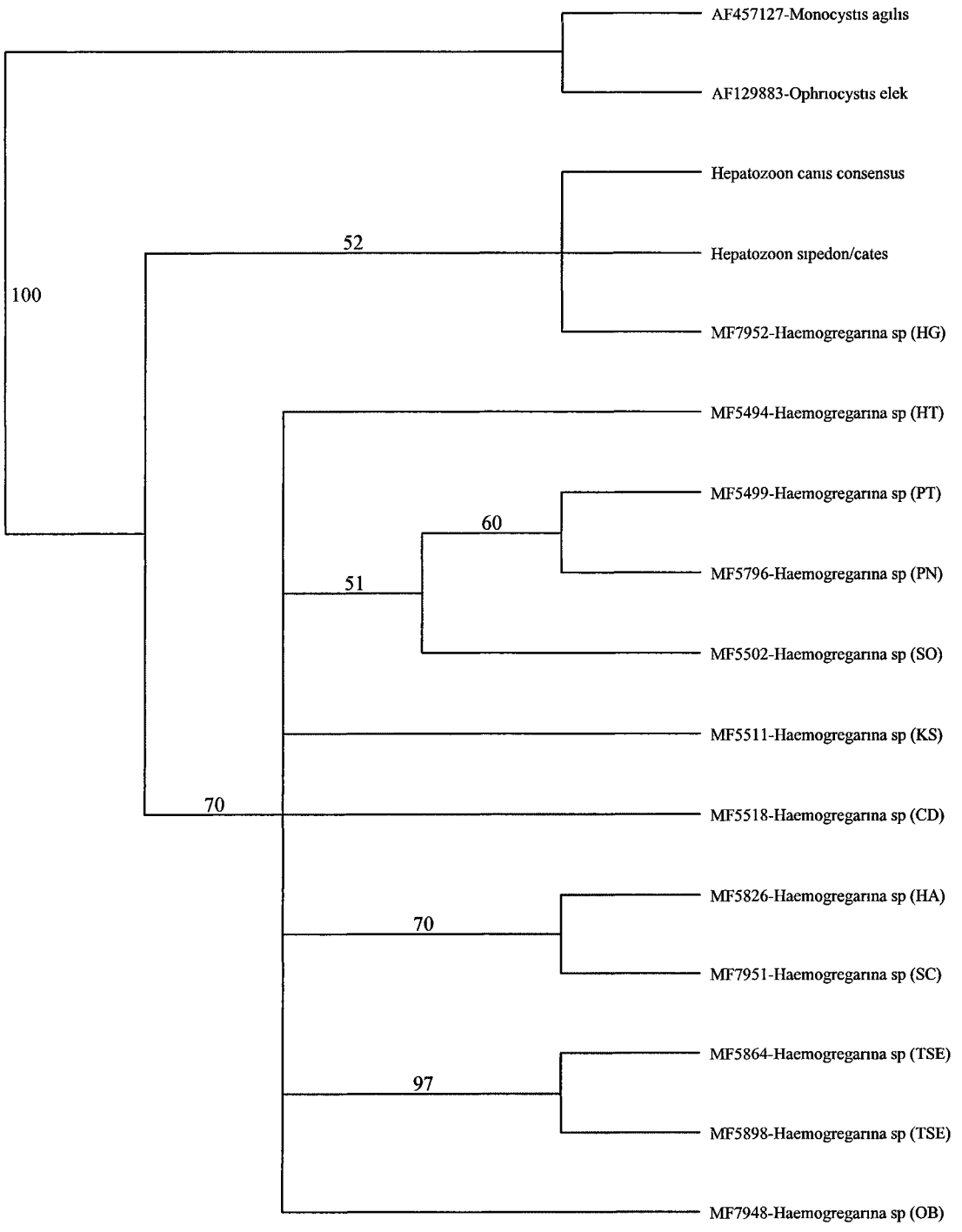


Figure F-43 Dataset C Bootstrap consensus tree by neighbor-joining with bootstrap values <50% support not shown. *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae and nucleotides were weighted equally With optimality criteria set to distance, a bootstrap neighbor-joining search was performed for 2500 replicates by random stepwise addition with TBR branch swap method The only strongly supported branch is the TSE-TSE clade The HA-SC clade is moderately supported *Haemogregarina* sp hosts are abbreviated as HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonorianse*, TSE=*Trachemys scripta elegans*

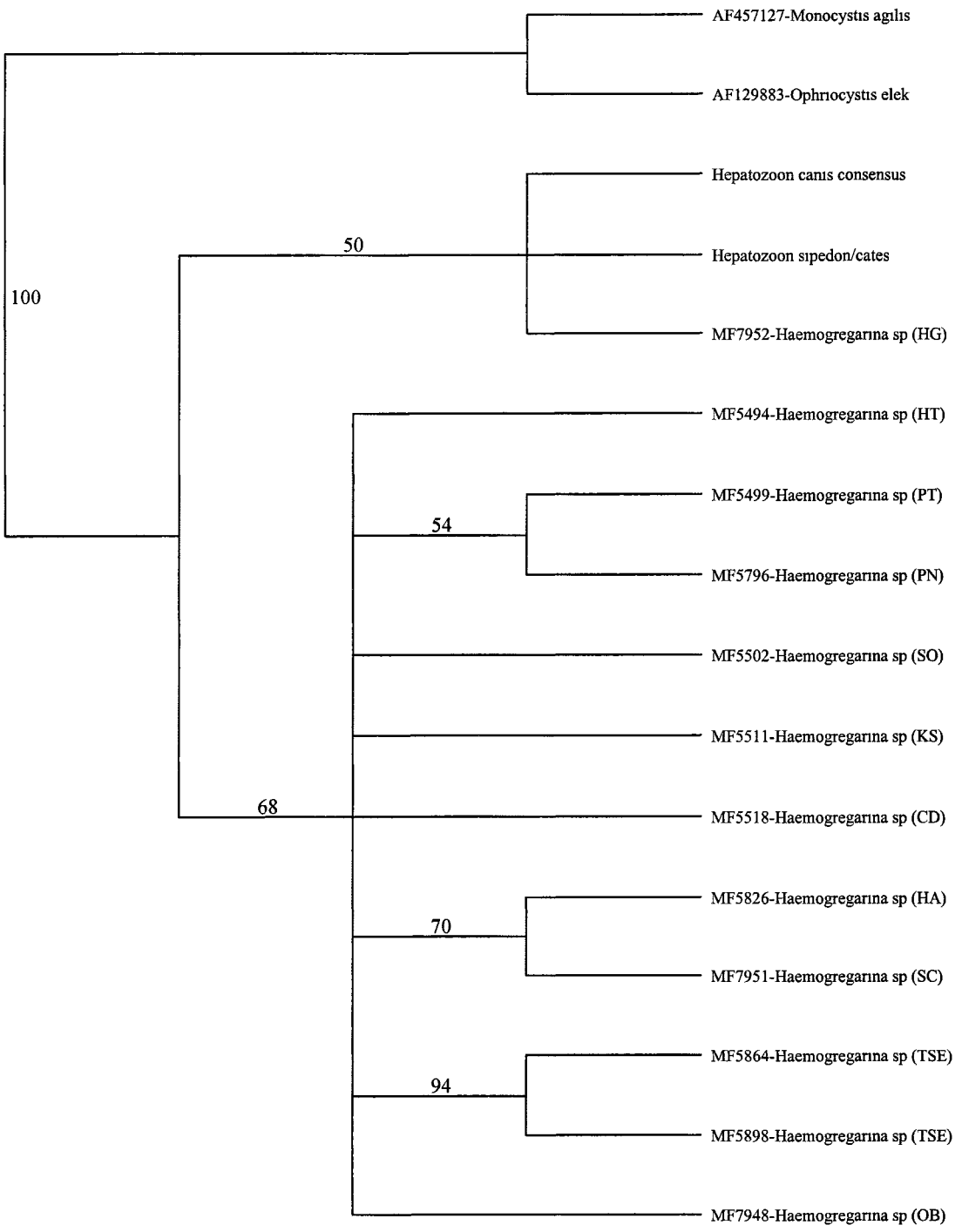


Figure F-44 Dataset C. Jackknife consensus tree by neighbor-joining with jackknife values <50% support not shown *Monocystis agilis* and *Ophriocystis elektroscirrha* were set as monophyletic outgroup to the Haemogregarinidae and nucleotides were weighted equally With optimality criteria set to distance, a jackknife neighbor-joining search was performed for 2500 replicates by random stepwise addition with TBR branch swap method The only strongly supported branch is the TSE-TSE clade. The HA-SC clade is moderately supported *Haemogregarina* sp hosts are abbreviated as: HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonoriense*, TSE=*Trachemys scripta elegans*.

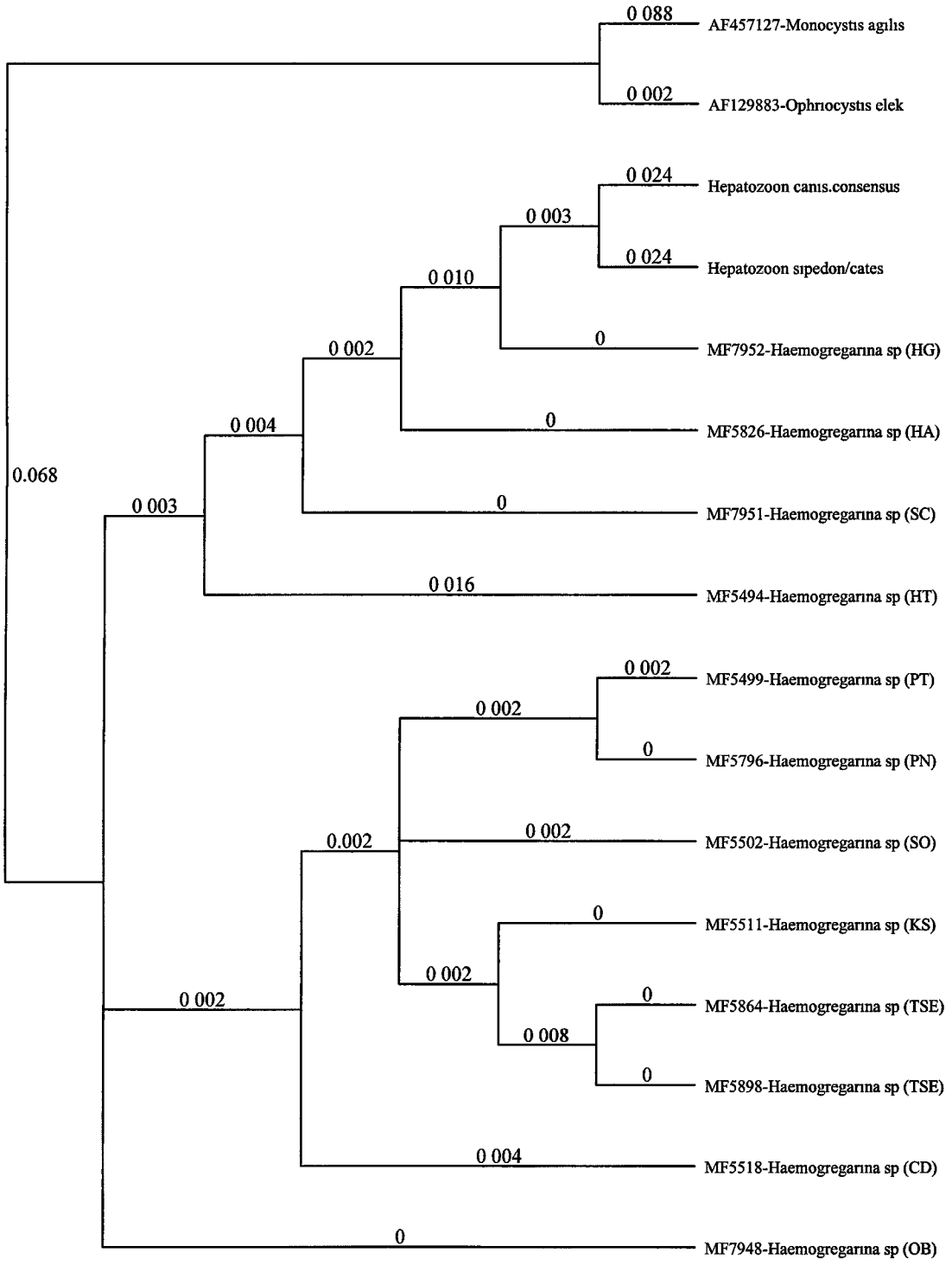


Figure F-45 Dataset C Maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters The parameters included model HKY + G; base frequencies of A=0 2826, C=0 1812, G=0 2432, T=0.2930; the Ti/Tv ratio set to 1 3000, ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2005 *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally With optimality criteria set to maximum likelihood, a heuristic search was performed with TBR branch swap method and ran until completion. *Haemogregarina* sp hosts are abbreviated as. HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonorianse*, TSE=*Trachemys scripta elegans*

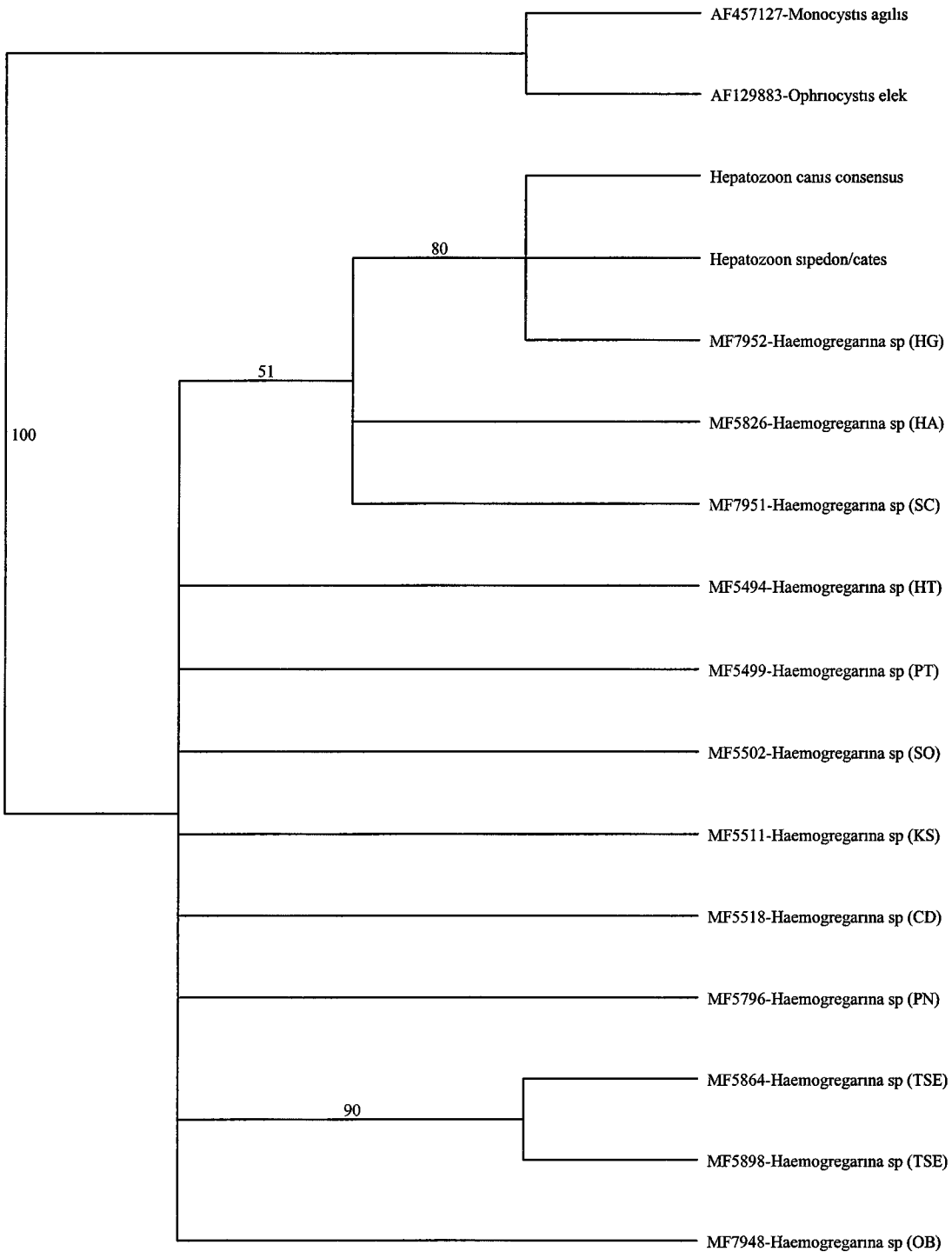


Figure F-46 Dataset C Bootstrap consensus for maximum likelihood using Modeltest parameters The parameters included model HKY + G, base frequencies of A=0 2826, C=0 1812, G=0 2432, T=0 2930, the Ti/Tv ratio set to 1 3000, ASRV invariable sites = 0 and variable sites with a gamma distribution of 0.2005 *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally. With optimality criteria set to maximum likelihood, a bootstrap heuristic search was performed for 2500 replicates by neighbor joining with TBR branch swap method Bootstrap values with <50% support are not shown *Haemogregarina* sp. hosts are abbreviated as. HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonorianse*, TSE=*Trachemys scripta elegans*

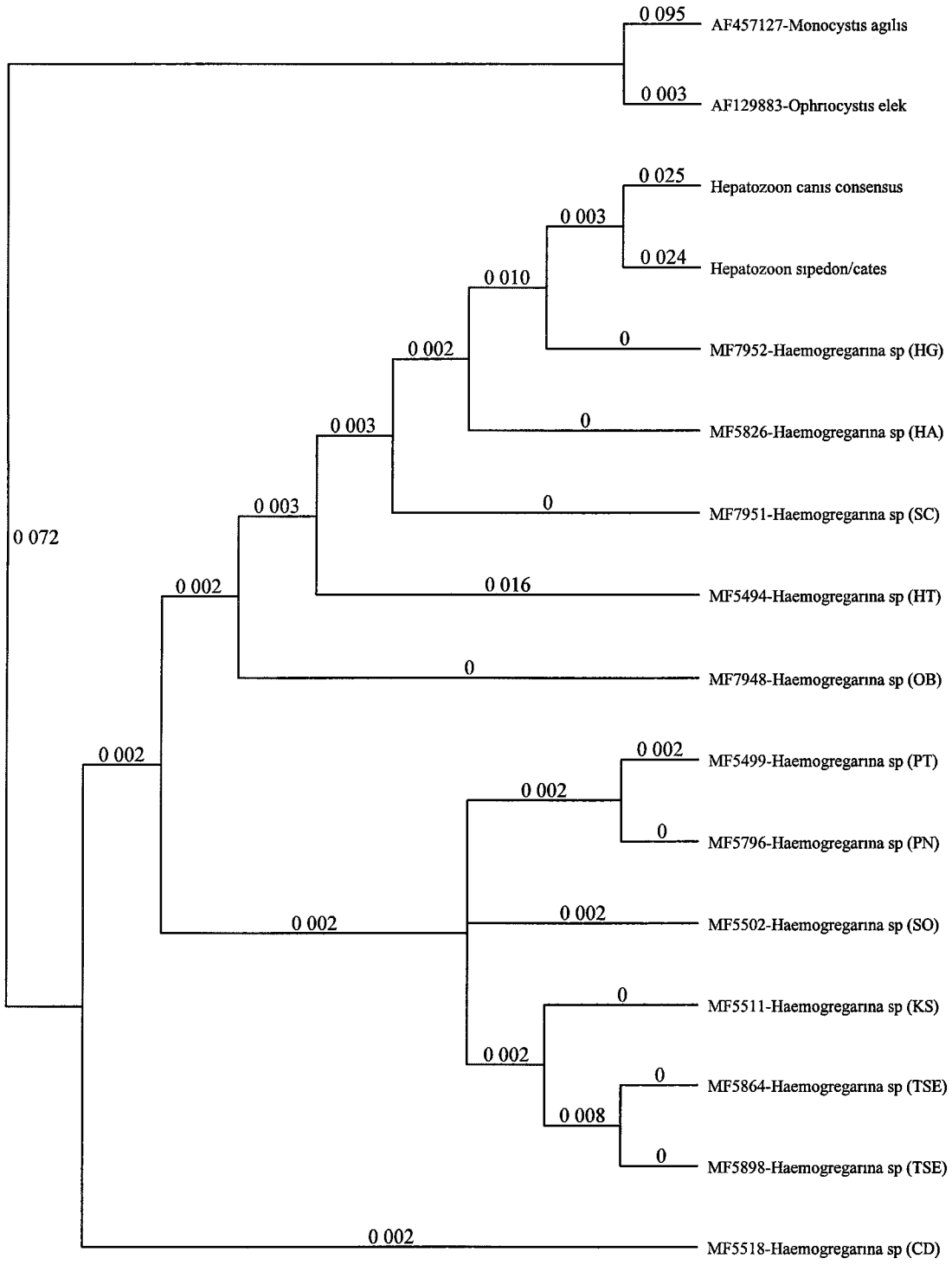


Figure F-47 Dataset C Maximum likelihood cladogram with branch lengths shown above branches using Modeltest parameters The parameters included model GTR + G, base frequencies of A=0 2798, C=0 1834, G=0 2582, T=0 2786, substitution rate matrix of A-C=0 9345, A-G=2 3842, A-T=2 2262, C-G=0 1839, C-T=4 2324, G-T=1 0000, ASRV invariable sites = 0 5390 and variable sites with a gamma distribution of 0 8563 *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally With optimality criteria set to maximum likelihood, a heuristic search was performed with TBR branch swap method and ran until completion The –ln L score is 1338 86140 *Haemogregarina* sp hosts are abbreviated as HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonoriense*, TSE=*Trachemys scripta elegans*

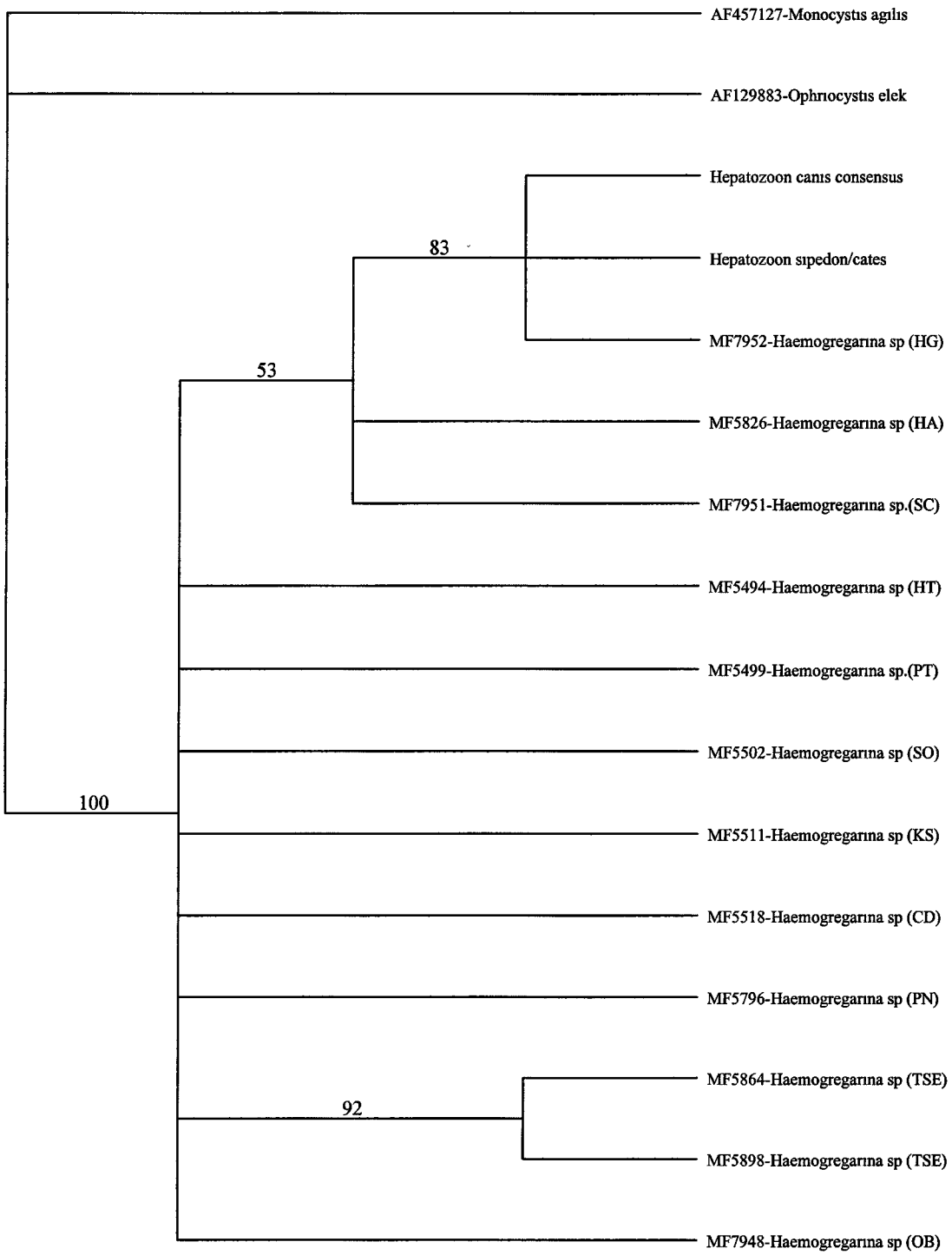


Figure F-48 Dataset C. Bootstrap consensus of maximum likelihood using Modeltest parameters The parameters included model GTR + G, base frequencies of A=0.2798, C=0 1834, G=0.2582, T=0 2786, substitution rate matrix of A-C=0.9345, A-G=2 3842, A-T=2 2262, C-G=0 1839, C-T=4.2324, G-T=1 0000, ASRV invariable sites = 0 5390 and variable sites with a gamma distribution of 0.8563 *Entamoeba histolytica* was set as outgroup to the Apicomplexa and nucleotides were weighted equally . With optimality criteria set to maximum likelihood, a bootstrap heuristic search was performed for 2500 replicates by neighbor joining with TBR branch swap method. Bootstrap values with <50% support are not shown *Haemogregarina* sp hosts are abbreviated as HA=*Hieremys annandalii*, HG=*Heosemys grandis*, SC=*Siebenrockiella crassicollis*, HT=*Hardella thurjii*, CD=*Cyclemys dentata*, OB= *Orlitia borneensis*, PT=*Pseudemys texana*, PN=*Pseudemys nelsoni*, SO=*Sternotherus odoratus*, KS=*Kinosternon sonoriense*, TSE=*Trachemys scripta elegans*

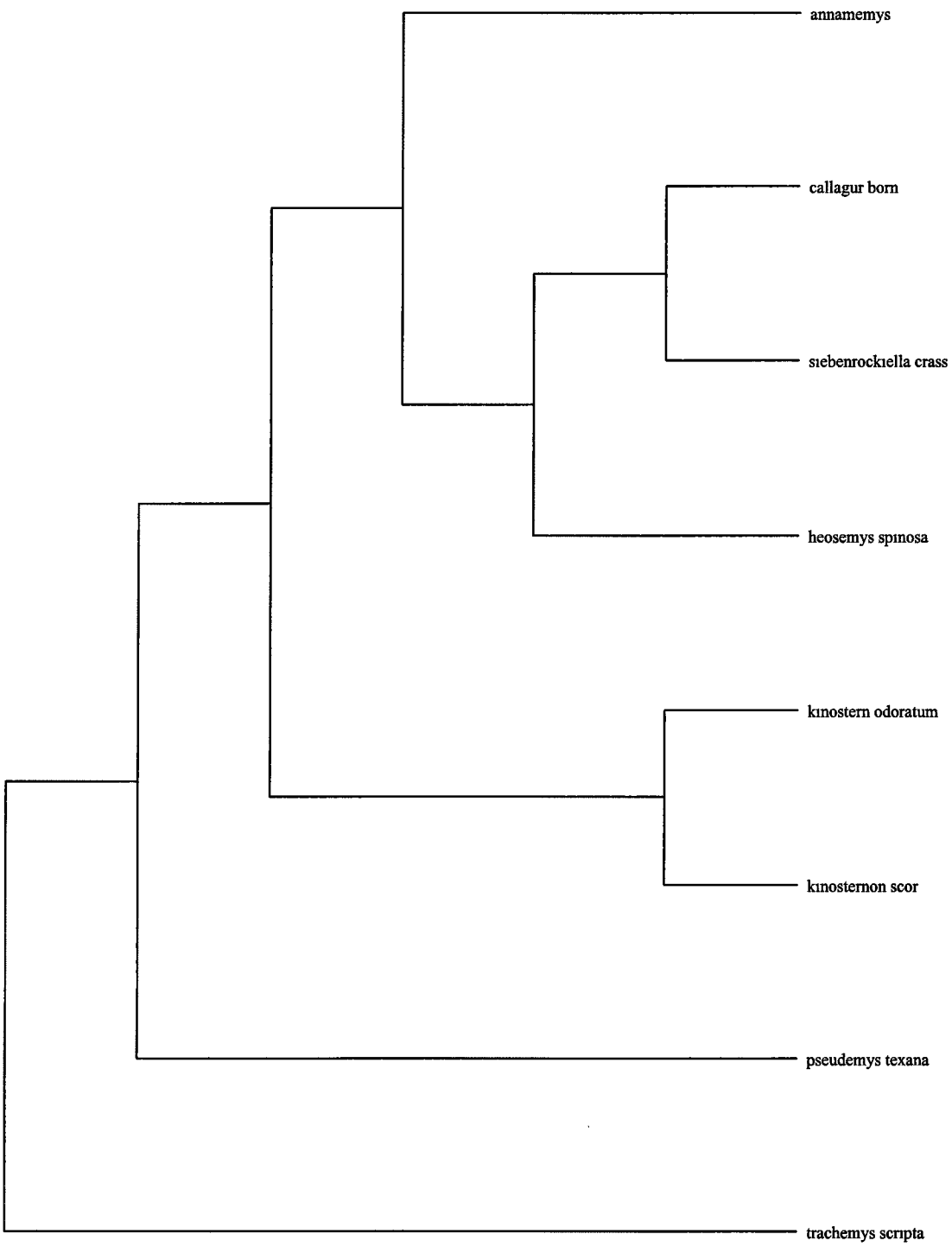


Figure F-49. Dataset D: The single most parsimonious tree with gaps treated a 5th base and nucleotides weighted equally. *Trachemys scripta* was set as outgroup to the ingroup. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. The most parsimonious tree had a TL=786, CI=0.763, RI=0.582, and RC=0.582.

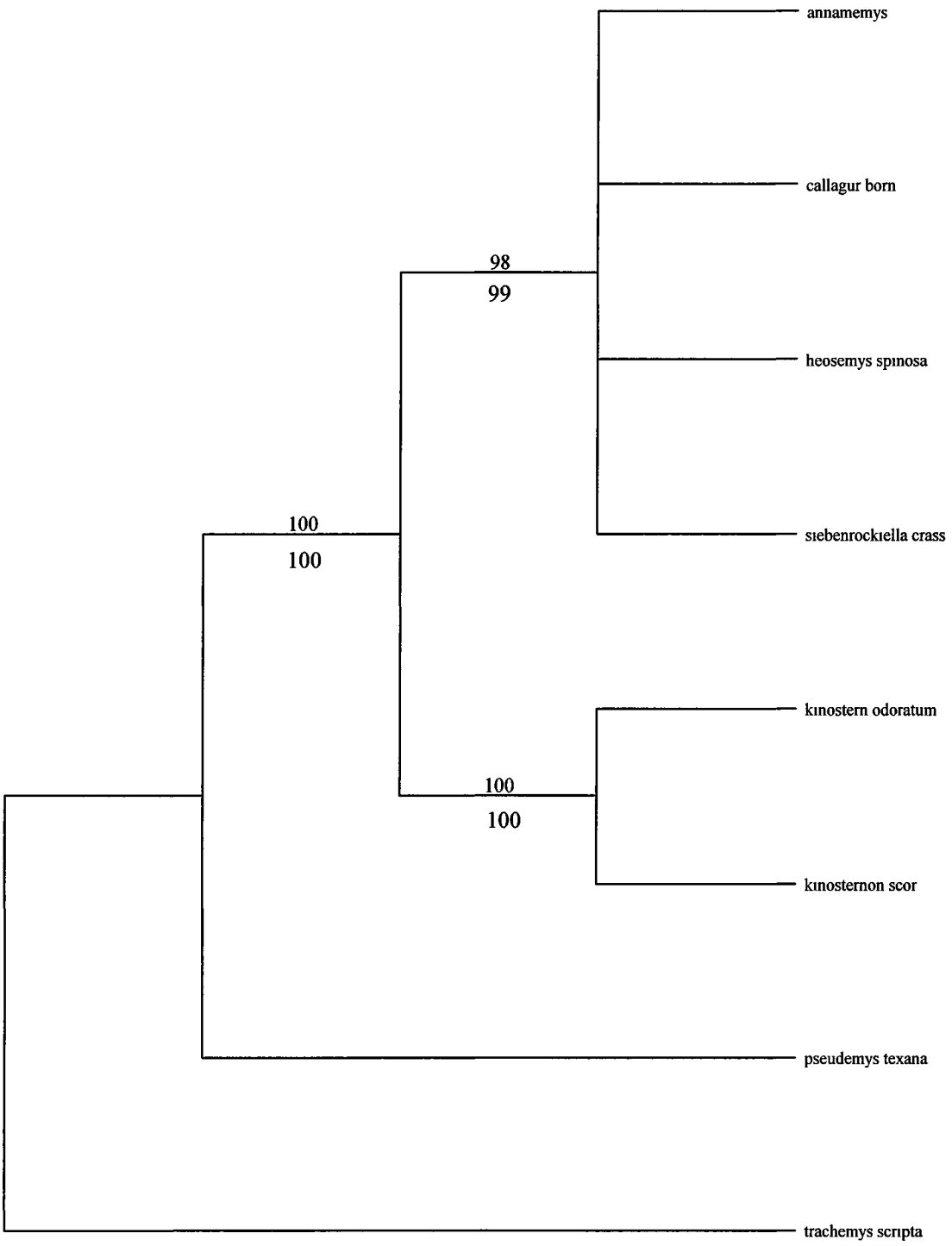


Figure F-50. Dataset D: Bootstrap and jackknife consensus tree by maximum parsimony with bootstrap and jackknife values <50% support not shown. Bootstrap values are represented above branches and jackknife values are represented below branches. *Trachemys scripta* was set as outgroup to the ingroup. With optimality criteria set to maximum parsimony, a bootstrap heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. Gaps were treated as 5th base and nucleotides weighted equally.

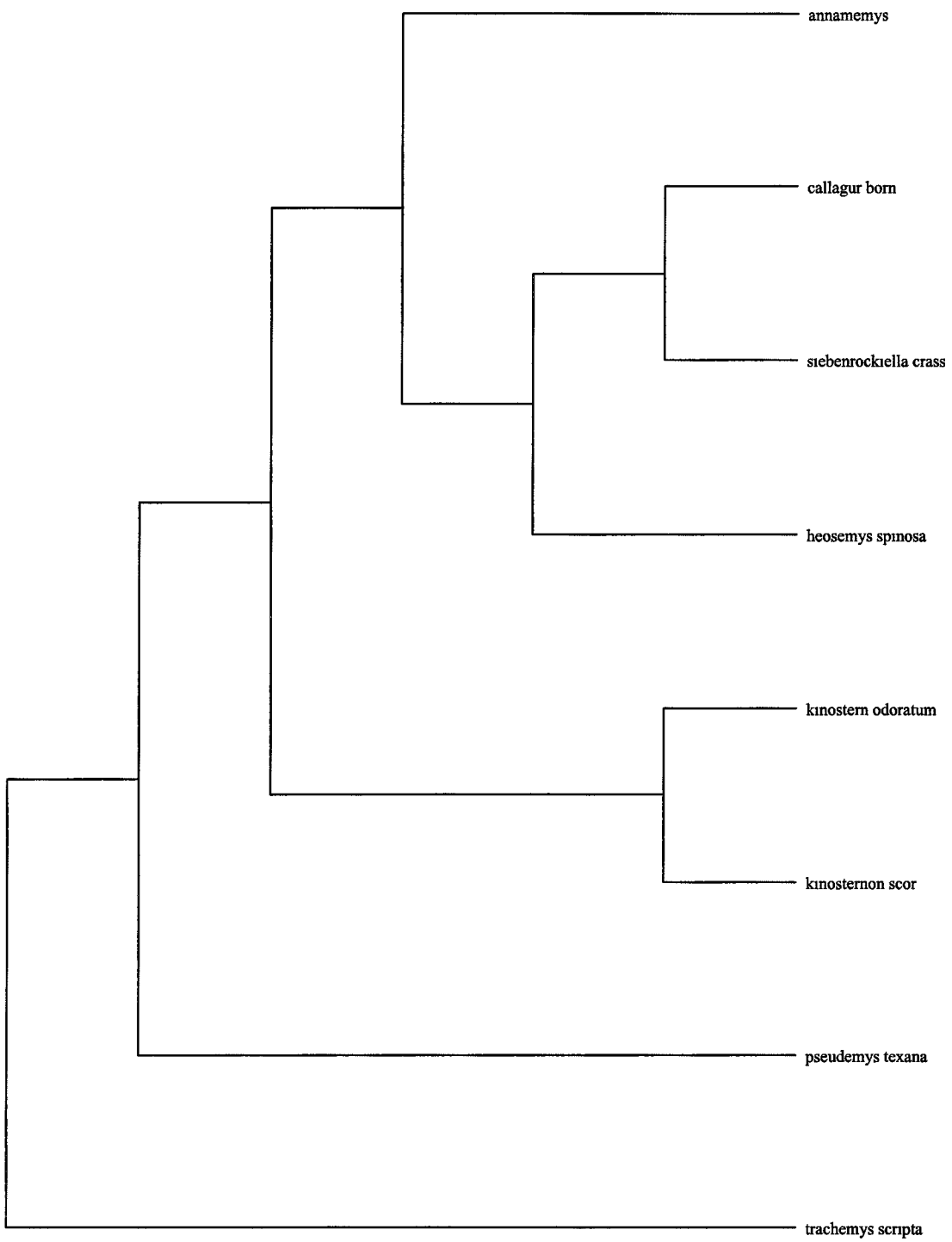


Figure F-51. Dataset D: The single most parsimonious tree with gaps treated a missing data and nucleotides weighted equally. *Trachemys scripta* was set as outgroup to the ingroup. With optimality criteria set to maximum parsimony, a heuristic search was performed for 1000 random replicates by random addition sequence with TBR branch swap method. The most parsimonious tree had a TL=707, CI=0.748, RI=0.571, and RC=0.427.

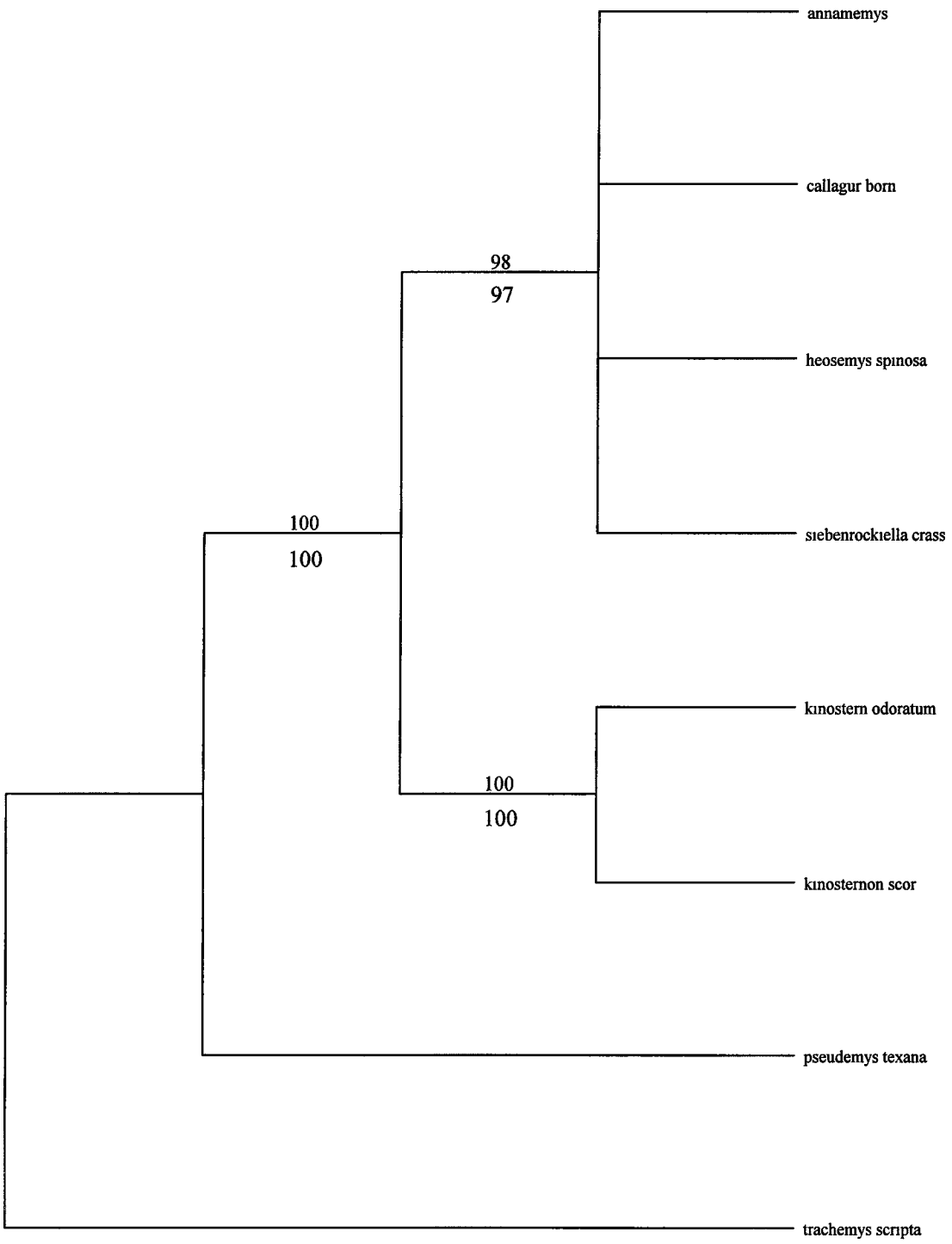


Figure F-52. Dataset D: Bootstrap and jackknife consensus tree by maximum parsimony with bootstrap and jackknife values <50% support not shown. Bootstrap values are represented above branches and jackknife values are represented below branches. *Trachemys scripta* was set as outgroup to the ingroup. With optimality criteria set to maximum parsimony, a bootstrap heuristic search was performed for 2500 replicates by random stepwise addition with TBR branch swap method. Gaps were treated as missing data and nucleotides were weighted equally.

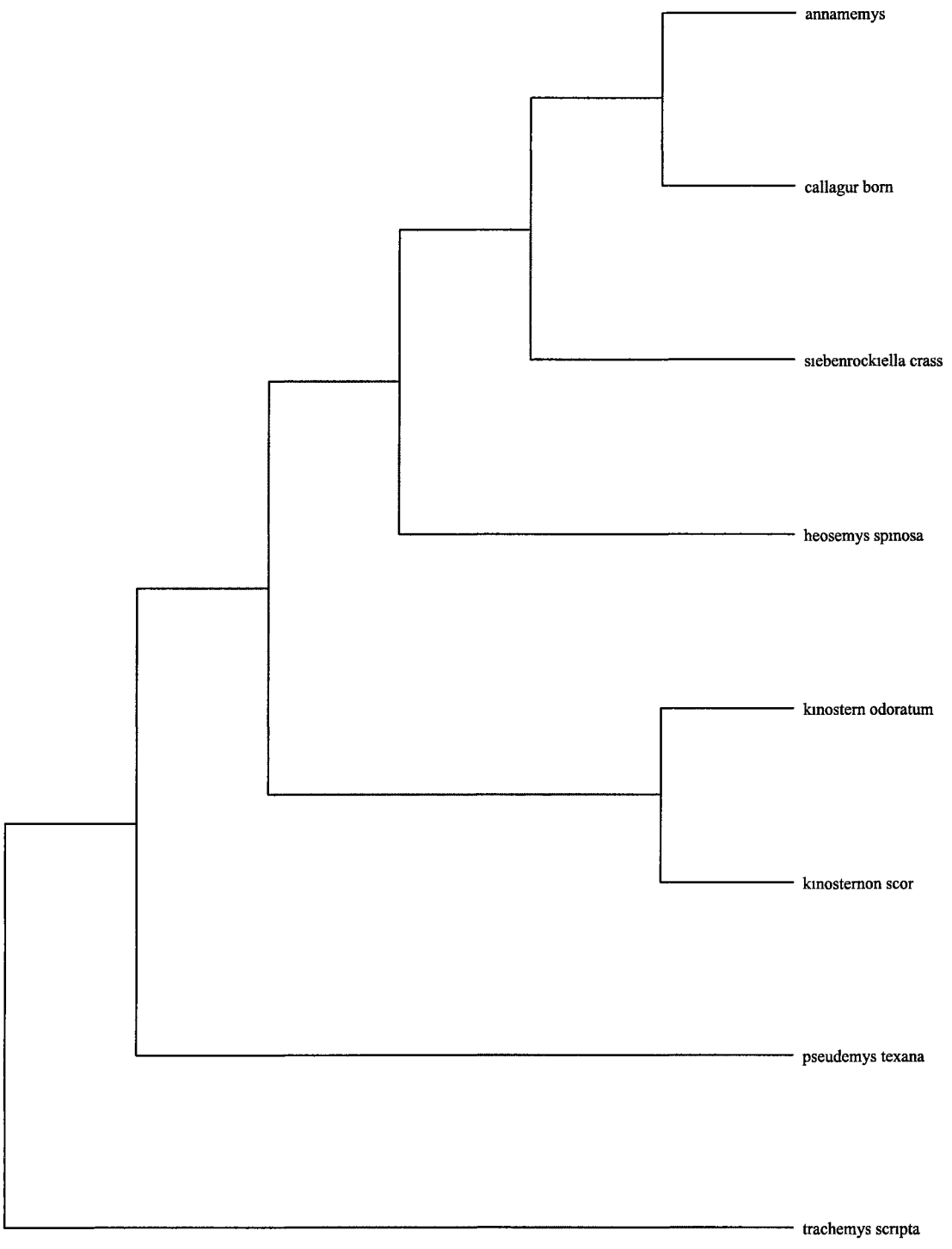


Figure F-53. Dataset D: Cladogram with optimality criterion set to distance with a neighbor joining search and HKY85 distance correction algorithm. *Trachemys scripta* was set as monophyletic outgroup to the ingroup and nucleotides were weighted equally. A neighbor joining search was performed for 1000 replicates by random stepwise addition with TBR branch swap method. The ME value is 0.75164

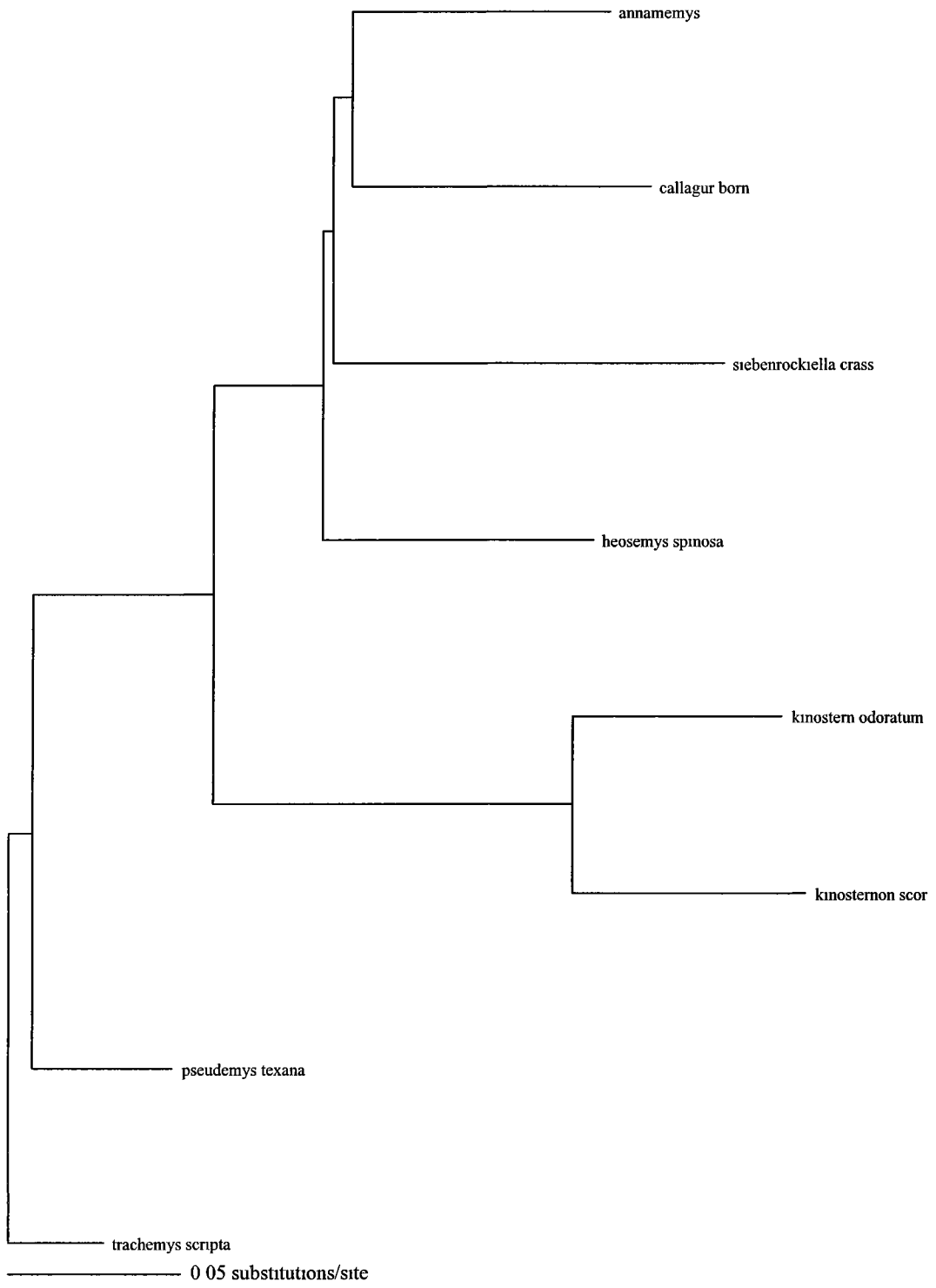


Figure F-54. Dataset D: Phylogram with optimality criterion set to distance with a neighbor joining search and HKY85 distance correction algorithm. *Trachemys scripta* was set as monophyletic outgroup to the ingroup and nucleotides were weighted equally. A neighbor joining search was performed for 1000 replicates by random stepwise addition with TBR branch swap method. The number of substitutions per site is represented as the length of each branch and the ME score is 0.75164 for the phylogram. This topology is identical to Figure F-53 however it is represented as a phylogram.

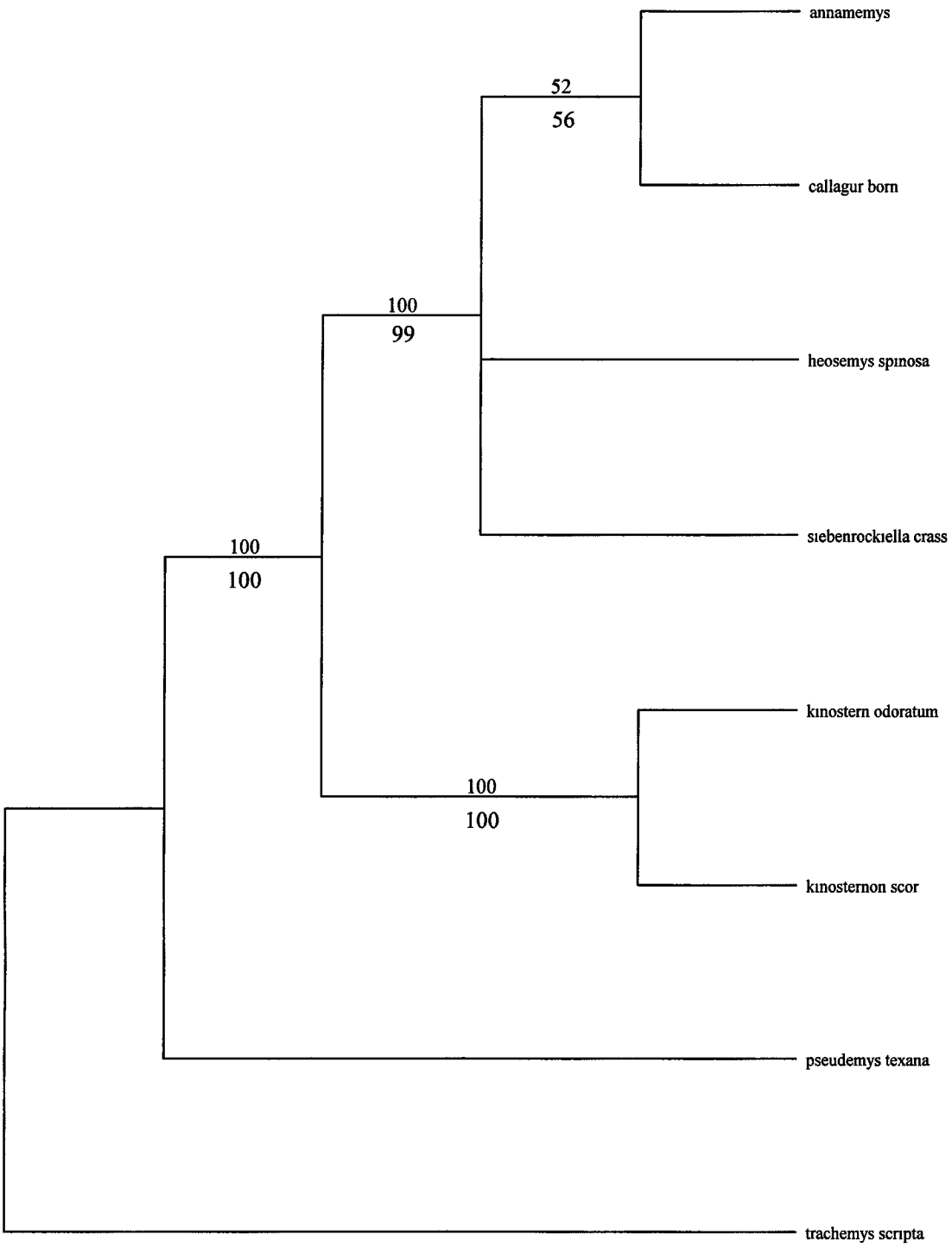


Figure F-55. Dataset D: Bootstrap and jackknife consensus tree by neighbor-joining with bootstrap and jackknife values <50% support not shown. Bootstrap values are represented above branches and jackknife values are represented below branches. *Trachemys scripta* was set as outgroup to the ingroup. With optimality criteria set to distance, a bootstrap neighbor-joining search was performed for 2500 replicates. Gaps were treated as missing data and nucleotides were weighted equally.

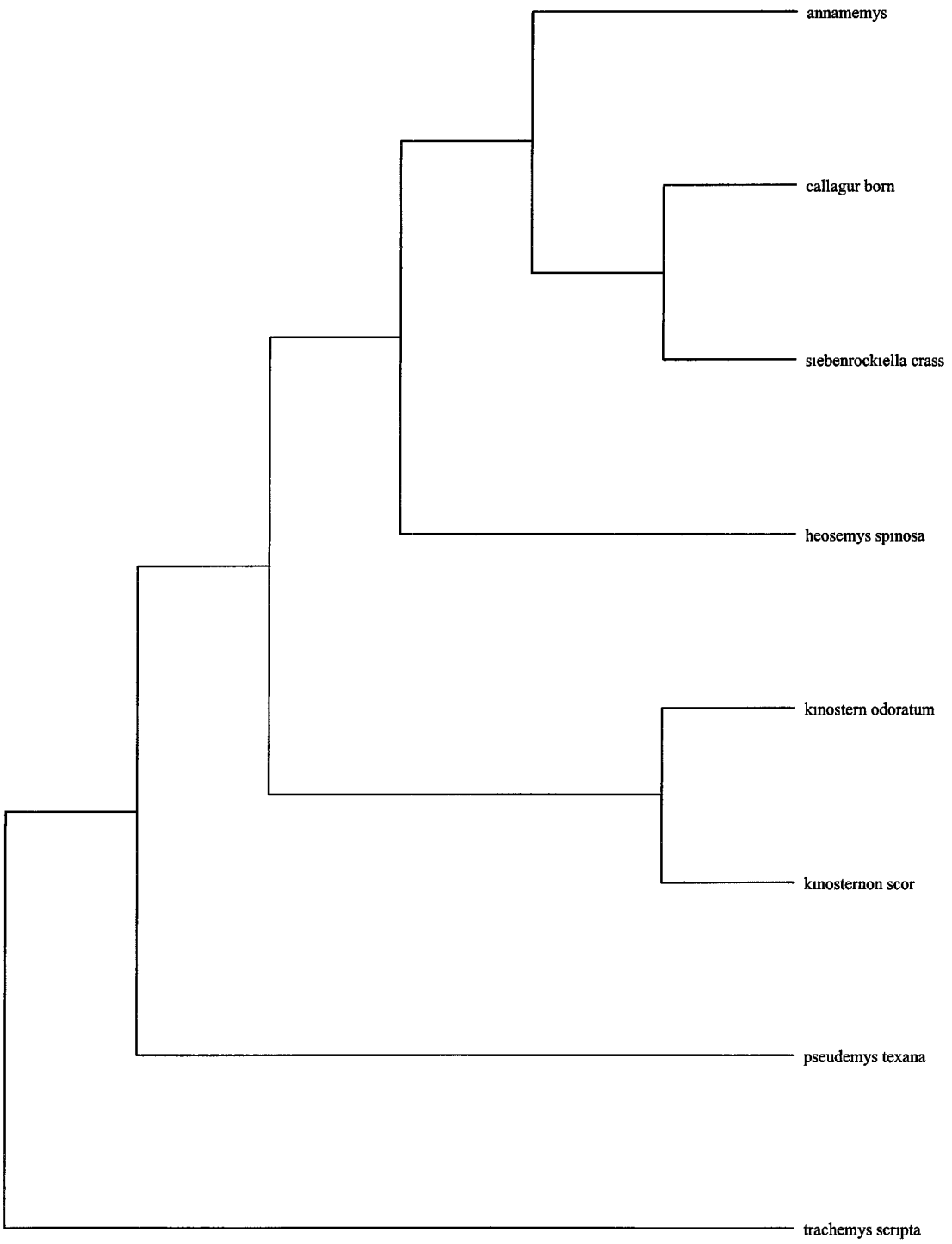


Figure F-56. Dataset D: Maximum likelihood using Modeltest parameters. The parameters included model GTR + G base frequencies of A=0.37230, C=0.26210, G=0.12320, T=0.24240; substitution rate matrix of A-C=2.040100, A-G=7.027900, A-T=0.963000, C-G=0.483500, C-T=15.696500, G-T=1.0000; ASRV invariable sites = 0, and variable sites with a gamma distribution of 0.3167. *Trachemys scripta* was set as outgroup to the ingroup and nucleotides were weighted equally.

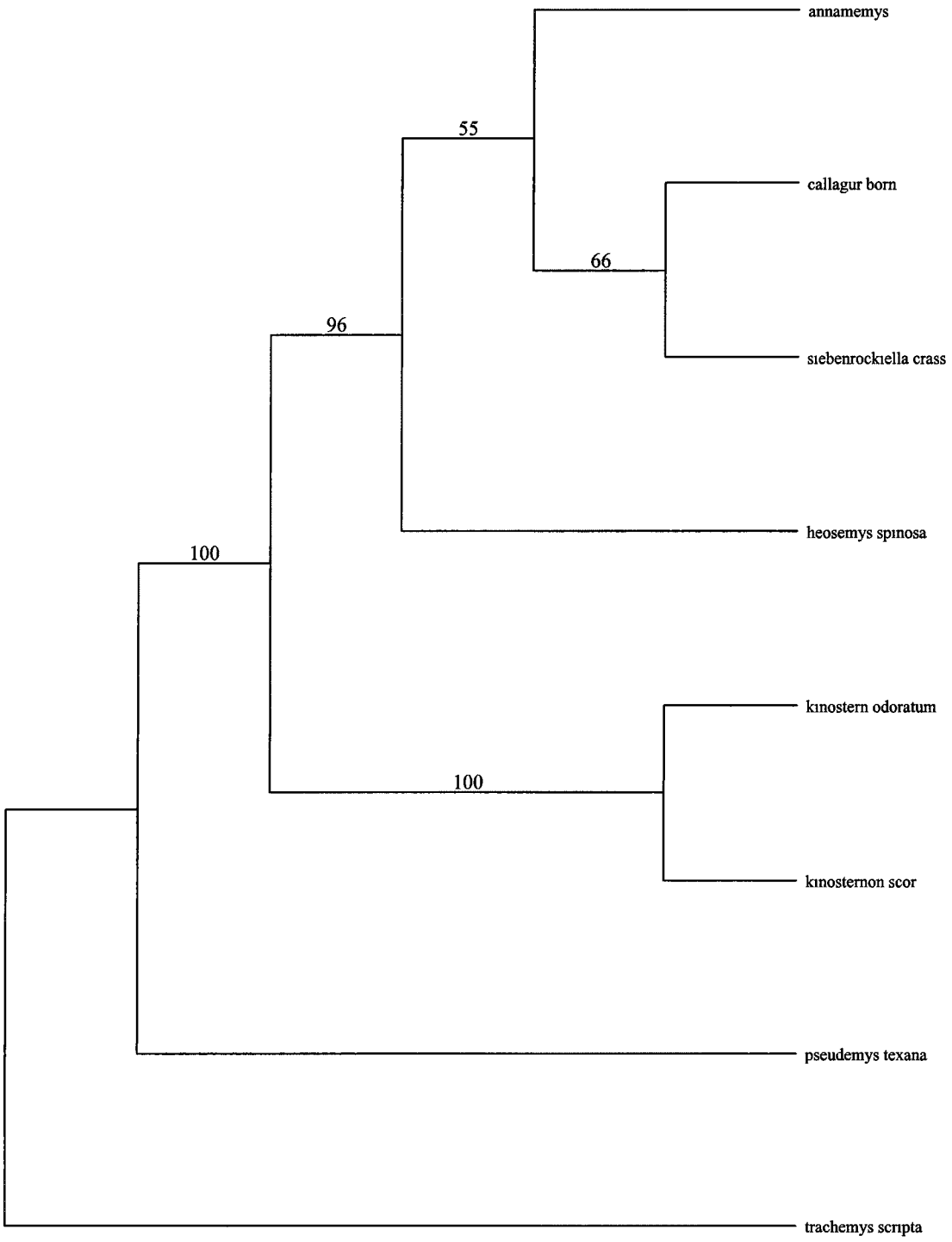


Figure F-57. Dataset D: Maximum likelihood using Modeltest parameters. The parameters included model GTR + G; base frequencies of A=0.37230, C=0.26210, G=0.12320, T=0 24240; substitution rate matrix of A-C=2.040100, A-G=7.027900, A-T=0.963000, C-G=0.483500, C-T=15.696500, G-T=1.0000; ASRV invariable sites = 0, and variable sites with a gamma distribution of 0.3167. *Trachemys scripta* was set as outgroup to the ingroup and nucleotides were weighted equally. Bootstrap values (>50%) are represented above each branch.

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