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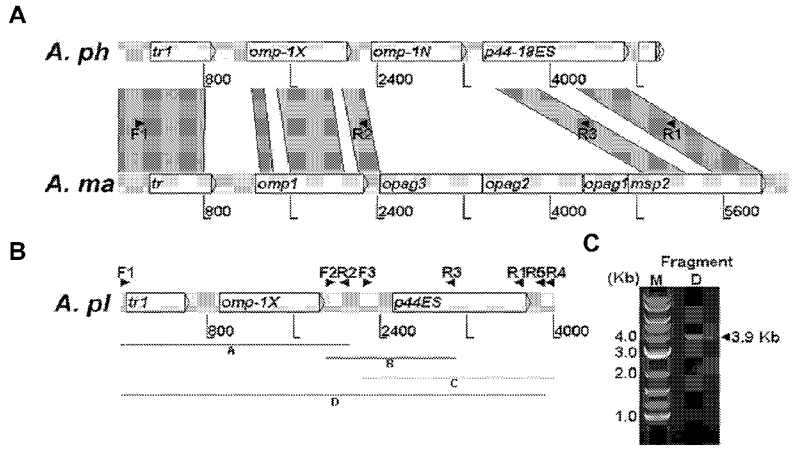


Figure 1

(57) Abstract: Described herein are improved diagnostic tools for veterinary and human use which can be used for serodiagnosing *A. platys* in mammals, particularly in members of the Canidae family and in humans. The diagnostic tools are a group of outer membrane proteins of *A. platys* and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of *A. platys* and variants thereof referred to hereinafter as the "P44 proteins", and antibodies to the OMP proteins and the P44 proteins.

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# COMPOSITIONS AND METHODS FOR THE DETECTION OF ANAPLASMA PLATYS

## GOVERNMENT RIGHTS

[0001] This work was sponsored in part by a National Institutes of Health Grant (R01AI47885). The United States government may have certain rights in the invention.

## BACKGROUND

[0002] *Anaplasma platys* (formerly *Ehrlichia platys*), an obligatory intracellular bacterium, was first described as a rickettsia-like agent in the platelets of dogs from Florida with infectious canine cyclic thrombocytopenia (ICCT) in 1978.[25] Authors pointed out morphological and biological similarity of this bacterium to *Ehrlichia canis* in infected dogs and *Anaplasma marginale* in infected cattle, two members of the family *Anaplasmataceae,*[25] which were well-known at that time. Clinical signs of ICCT are fever, depression, appetite loss, anorexia, and bleeding tendencies.[22] Parasitemia and thrombocytopenia occur in cycles at approximately 10 to 14 day intervals.[22] *Anaplasma platys* responds well to doxycycline, a tetracycline antibiotic, as the primary means of treatment.

[0003] Based on indirect fluorescence antibody (IFA) tests using the platelet-rich plasma from a dog experimentally infected with ICCT, minimal serologic cross-reaction was found to occur between *A. platys* and *E. canis,* and the researchers proposed the name "*Ehrlichia platys*" for this bacterium.[22] In 1992, the 16S rRNA gene sequence of *A. platys* was reported.[3] Subsequently, the *groEL* gene sequence of *A. platys* was disclosed.[29, 67] Phylogenetic analysis of these sequences showed that this is a distinct bacterium closely related to *Anaplasma phagocytophilum* and *Anaplasma marginale*, which led to reclassification of this bacterium into the genus *Anaplasma*.[17] Later it was reported that although *A. platys* does not cross-react with serum antibodies from dogs infected with *E. canis* on IFA tests, the *A. platys* antigen cross-reacts with anti-*Anaplasma phagocytophilum* antibodies.[32]

[0004] Seropositive dogs have been found in Florida, Pennsylvania, Texas, Louisiana, Illinois, California, Arkansas, Mississippi, Idaho, and North Carolina. High rates of *A. platys* and *E. canis* dual positive dogs have also been reported throughout these areas.[22] *A.*

*platys* DNA has also been detected in dogs throughout Brazil,[20] Greece,[43] France,[33] Taiwan,[15] Spain,[54] China,[28] Australia,[12] Portugal,[13] the Democratic Republic of Congo,[55] Japan,[61] Thailand,[29] and Venezuela.[59] It is believed that the brown dog tick, *Rhipicephalus sanguineus*, is the biological vector which transfers *A. platys* to potential hosts. In fact, *A. platys* has been detected in brown dog ticks in Okinawa, Japan,[34] Spain,[58] and the Democratic Republic of Congo.[55] However, it has not been experimentally proven that *R. sanguineus* is the biological vector responsible for the transfer of *A. platys*.[56] To date, *A. platys* has never been culture isolated. Consequently this bacterium is poorly understood at the molecular, cellular, or immunologic level, and to date, no antigen has been identified for this bacterium.

[0005] In *A. phagocytophilum* and *A. marginale,* surface-exposed immunodominant 44 kDa major outer membrane proteins (P44s/Msp2s) are encoded by the *p44* (*msp2*) polymorphic multigene family.[6, 9, 39, 41, 69-71] In *A. phagocytophilum,* P44 proteins consist of a single central hypervariable region of approximately 94 amino acid residues and an N-terminal and C-terminal conserved regions of approximately 186 and 146 amino acid residues, respectively.[41] A single polymorphic *p44/msp2* expression locus (*p44/msp2ES*) is found in the genome of *A. phagocytophilum*[10] and *A. marginale*,[26] respectively. Both expression loci are found downstream of *tr1* genes encoding putative transcriptional factor and homologs of *Ehrlichia chaffeensis omp-1* genes encoding polymorphic major outer membrane protein (MOMP).[6, 8, 39] At *p44/msp2ES, p44*s and *msp2* donor sequences elsewhere in the genome undergo recombination via RecF pathway to allow variable *p44/msp2ES* expression under the same promoter.[6, 8, 39, 40] This mechanism is thought to facilitate P44/Msp2 antigenic variation persistent infection and for adaptation to new environments such as transmission between tick and mammalian hosts.[7, 11, 38, 40, 65, 71] Purified native P44 from *A. phagocytophilum* and purified native OMP-1s (P28 and OMP-1F) of *Ehrlichia chaffeensis* have porin activity.[30, 37]

[0006] *Anaplasma platys* (Apl) is an obligate intracellular bacteria that infects platelets and causes a cyclic thrombocytopenia in the dog. The observation than a dog can be affected by this rickettsial agent, and the disease is most likely transmitted by the *Rhipicephalus spp* of ticks. *Anaplasma platys* was first reported in the United States in 1978 and has since been reported in Europe, Asia, South America, the Middle East, Australia, and Africa. Because of the common vector, *Anaplasma platys* infection is often found as a co-

infection with *Ehrlichia canis*. The ability of the organism to produce clinical disease in the dog appears to vary with geography, suggesting that strain differences may contribute to virulence. *Anaplasma platys* is related to another Anaplasma species known to cause clinical disease in the dog, *Anaplasma phagocytophilum* (Aph).

[0007] Current diagnostic tests that attempt to distinguish Aph and *Anaplasma platys* have limited specificity. PCR for Aph and *Anaplasma platys* using 16SrRNA has also had problems with specificity. Therefore, assays for specific detection of *Anaplasma platys* are needed in the art. Additionally, serological tests for *Anaplasma platys* are also needed.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0008] **Figure 1** shows the (A) strategy for *A. platys p44ES* cluster sequencing. *A. phagocytophilum p44ES* and *A. marginale msp2ES* were aligned to design primer F1 (targeting *tr1/orf3* upstream highly conserved region) and degenerate primers R1 (targeting *p44ES/msp2* C-terminal highly conserved region), R2 (targeting conserved intergenetic region between *omp-1X/omp4* and *omp-1N/omp3*), R3 (targeting *p44ES/msp2* N-terminal conserved region) and R4 (based on *p44ES/msp2ES* downstream conserved *valS* gene). Primers F2 and F3 were designed based on the sequence results. (B) The final sequence (4,009 bp) was assembled with the SeqMan program from the DNASTAR software suite. Genes are represented as boxes with arrows indicating their orientation. (C) The entire expression locus fragment D (arrow) amplified from the dog 2 blood DNA specimen by primers F1 and R5.

[0009] **Figure 2** depicts the synteny analysis, using the Artemis comparison tool, of the *A. platys* (*A. pl*) *p44ES* cluster relative to *A. phagocytophilum* (*A. ph*) and *A. marginale* (*A. ma*). Each bar corresponds to a good match. Numbers indicate bp. Score cutoffs: 140.

[0010] **Figure 3** depicts the synteny analysis, using the Artemis comparison tool, of the *A. platys* (*A. pl*) *p44ES* cluster relative to *A. phagocytophilum* (*A. ph*) and *A. marginale* (*A. ma*). Each bar corresponds to a good match. Numbers indicate bp. Score cutoffs: 140 using the Artemis Comparison Tool.

[0011] **Figure 4** shows the hylogenetic tree of OMP-1X proteins of *A. platys*, *A. phagocytophilum*, *A. marginale*, *E. canis*, *E. chaffeensis*, *E. ewingii*, *E. ruminantium*. The tree was constructed using DNASTAR MegAlign Clustal W method.

[0012] **Figure 5** shows the Tr1 sequence alignment of *A. platys, A. phagocytophilum,* and *A. marginale.*

[0013] **Figure 6A** shows the phylogram of Tr1 proteins of *A. platys*, *A. phagocytophilum*, and *A. marginale*. The tree was constructed using DNASTAR MegAlign Clustal W method. **Figure 6B** shows the amino acid sequences identity between *A. platys* Tr1 and *A. phagocytophilum* Tr1 (YP_505749) or *A. marginale* AM1138 (YP_154239) were 86.4% and 73.1%, respectively.

[0014] **Figure 7** shows the OMP-1X sequence alignment of *A. platys, A. phagocytophilum,* and *A. marginale.*

[0015] **Figure 8A** shows the phylogram of OMP-1X proteins of *A. platys*, *A. phagocytophilum*, and *A. marginale*. The tree was constructed using DNASTAR MegAlign Clustal W method. **Figure 8B** shows the amino acid sequence identities between *A. platys* OMP-1X and *A. phagocytophilum* OMP-1X (YP_505750) or *A. marginale* outer membrane protein 1 (YP_154240) were 42.3% and 39.5%, respectively.

[0016] **Figure 9** shows the P44 sequence alignment of *A. platys, A. phagocytophilum,* and *A. marginale.*

[0017] **Figure 10A** shows the phylogram of P44 proteins of *A. platys*, *A. phagocytophilum*, and *A. marginale*. The tree was constructed using DNASTAR MegAlign Clustal W method. **Figure 10B** shows the amino acid sequences identity between *A. platys* P44ES and *A. phagocytophilum* P44-18ES (YP_505752) or *A. marginale msp2* (YP_154245) were 55.0~56.9% and 41.5~42.1%, respectively.

[0018] **Figures 11A and 11B** show P44ES/Msp2 proteins of *A. platys, A. phagocytophilum,* and *A. marginale.* A total of 24 P44/Msp2s were segregated into 3 clusters. The tree was constructed using the Neighbor-Joining (NEIGHBOR program from PHYLIP) method based on the alignment generated with Clustal V; 1,000 bootstrap replications were performed. The nodes supported by bootstrap values greater than 60% are labeled.

[0019] **Figure 13** shows the sequence alignment was completed using a DNASTAR SeqMan program. Alignment of *A. platys* (*A.pl*) OMP-1X protein with related proteins from

*A. phagocytophilum* (*A.ph*), *A. marginale* (*A.ma*), *E. canis* (*E.ca*), *E. chaffeensis* (*E.ch*), *E. ewingii* (*E. ew*), and *E. ruminantium* (*E.ru*) using the Clustal W method revealed a unique region in *A. platys* (AVQEKKPPEA (SEQ ID NO: 98), box lined by dashed bar). The antigenic index and surface probability profile suggest that this region is both antigenic and surface-exposed.

[0020] **Figure 14** shows the ELISA analysis of samples from *A. platys* PCR-positive dogs (No. 1-3), *A. platys* PCR-negative dogs (No. 4-6), and *A. phagocytophilum* seropositive horse serum samples (No. 7-9) with the *A. platys* specific peptide. The *y* axis shows the $OD_{415}$-$OD_{492}$ values. A reaction was considered positive when the plasmas from infected dogs yielded an $OD_{415}$-$OD_{492}$ value greater than the mean $OD_{415}$-$OD_{492}$ value for negative control plasma plus 3 standard deviations (dashed line). Representative data from triplicate assays are shown.

[0021] **Figure 15** shows the alignment of *A. platys, A. phagocytophilum,* and *A. marginale* p44/msp2 DNA. *A. platys*-species specific primers useful for species-specific PCR diagnosis are underlined in bold.

[0022] **Figure 16** shows the *A. platys, A. phagocytophilum,* and *A. marginale* amino acid sequence alignment.

[0023] **Figure 17** shows the complete sequence assembly for the *A. platys* expression locus.

[0024] **Figure 18** shows the *A. platys* P44 alignment (DNA and protein).

[0025] **Figures 19A and 19B** show the *A. platys*-specific P44 sequence, distinct from *A. phagocytophilum* and *A. marginale* or IDEXX P44 partial sequences useful for species-specific serodiagnosis.

[0026] **Figure 20** shows the comparison of the *A. platys*-specific P44 sequence to the sequences of *A. phagocytophilum* and *A. marginale*.

[0027] **Figure 21** shows the *A. platys*-specific primer regions and sequences from dog 2.

[0028] **Figure 22** shows the sequence alignment was completed using a DNASTAR SeqMan program. Alignment of *A. platys* (*A.pl*) OMP-1X protein with related proteins from

*A. phagocytophilum* (*A.ph*), *A. marginale* (*A.ma*), *E. canis* (*E.ca*), *E. chaffeensis* (*E.ch*), *E. ewingii* (*E. ew*), and *E. ruminantium* (*E.ru*) using the Clustal W method revealed a unique region in *A. platys* (AVQEKKPPEA (SEQ ID NO: 98). The antigenic index and surface probability profile suggest that this region is both antigenic and surface-exposed, and distinct from those of other species. Figure 22 also shows the identification of two regions in *A. platys* that are both antigenic and surface-exposed, and distinct from those of other species. These two regions are identified in boxes as "1" and "2".

[0029] **Figure 23** shows the sequence alignment was completed using a DNASTAR SeqMan program. Alignment of *A. platys* (*A.pl*) p44ES protein with related proteins from *A. phagocytophilum* (*A.ph*), *A. marginale* (*A.ma*), *E. canis* (*E.ca*), *E. chaffeensis* (*E.ch*), *E. ewingii* (*E. ew*), and *E. ruminantium* (*E.ru*) using the Clustal W method revealed regions in *A. platys* wherein the antigenic index and surface probability profile suggest that these regions are both antigenic and surface-exposed, and distinct from other species. These regions are identified in boxes as "1" through "6" as well as in brackets under the antigenic index and surface probability profile.

## DETAILED DESCRIPTION

[0030] Described herein are improved diagnostic tools for veterinary and human use which can be used for serodiagnosing *A. platys* in mammals, particularly in members of the *Canidae* family and in humans. The diagnostic tools are a group of outer membrane proteins of *A. platys* and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of *A. platys* and variants thereof referred to hereinafter as the "P44 proteins", and antibodies to the OMP proteins and the P44 proteins.

[0031] The present invention can be understood more readily by reference to the following detailed description of the invention and the Examples included therein.

[0032] All patents, patent applications, and publications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entireties into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

[0033] Unless otherwise expressly stated, it is in no way intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a specific

order. Accordingly, where a method claim does not specifically state in the claims or descriptions that the steps are to be limited to a specific order, it is in no way intended that an order be inferred, in any respect. This holds for any possible non-express basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, or the number or type of aspects described in the specification.

**Definitions**

[0034] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The terminology used in the description of the embodiments herein is for describing particular embodiments only and is not intended to be limiting of the embodiments disclosed. As used in the description, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

[0035] Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in this disclosure are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in this disclosure are approximations that may vary depending upon the desired properties sought to be obtained by the present disclosure. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of any claims, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[0036] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values described herein, and that each value is also herein disclosed as "about" that particular

value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that when a value is disclosed that "less than or equal to" the value, "greater than or equal to the value" and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value "10" is disclosed the "less than or equal to 10" as well as "greater than or equal to 10" is also disclosed. It is also understood that throughout the application, data are provided in a number of different formats, and that these data, represent endpoints, starting points, and ranges for any combination of the data points. For example, if a particular data point "10" and a particular data point 15 are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed as well as between 10 and 15. It is also understood that each unit between two particular units is also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[0037] The term "subject" means an individual. In one aspect, a subject is a mammal such as a primate, and, more preferably, a human. Non-human primates include marmosets, monkeys, chimpanzees, gorillas, orangutans, and gibbons, to name a few. The term "subject" also includes domesticated animals, such as cats, dogs, etc., livestock (for example, cattle (cows), horses, pigs, sheep, goats, etc.), laboratory animals (for example, ferret, chinchilla, mouse, rabbit, rat, gerbil, guinea pig, etc.) and avian species (for example, chickens, turkeys, ducks, pheasants, pigeons, doves, parrots, cockatoos, geese, etc.). Subjects can also include, but are not limited to fish (for example, zebrafish, goldfish, tilapia, salmon, and trout), amphibians and reptiles. As used herein, a "subject" is the same as a "patient," and the terms can be used interchangeably.

[0038] As used herein, the term "amino acid sequence" refers to a list of abbreviations, letters, characters or words representing amino acid residues. The amino acid abbreviations used herein are conventional one letter codes for the amino acids and are expressed as follows: A, alanine; C, cysteine; D aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H histidine; I isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine..

[0039] "Polypeptide" as used herein refers to any peptide, oligopeptide, polypeptide, gene product, expression product, or protein. A polypeptide is comprised of consecutive amino

acids. The term "polypeptide" encompasses naturally occurring or synthetic molecules. The terms "polypeptide," "peptide," and "protein" can be used interchangeably.

[0040] In addition, as used herein, the term "polypeptide" refers to amino acids joined to each other by peptide bonds or modified peptide bonds, e.g., peptide isosteres, etc. and may contain modified amino acids other than the 20 gene-encoded amino acids. The polypeptides can be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. The same type of modification can be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide can have many types of modifications. Modifications include, without limitation, acetylation, acylation, ADP-ribosylation, amidation, covalent cross-linking or cyclization, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of a phosphytidylinositol, disulfide bond formation, demethylation, formation of cysteine or pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristolyation, oxidation, pergylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, and transfer-RNA mediated addition of amino acids to protein such as arginylation. (See Proteins – Structure and Molecular Properties 2nd Ed., T.E. Creighton, W.H. Freeman and Company, New York (1993); Posttranslational Covalent Modification of Proteins, B.C. Johnson, Ed., Academic Press, New York, pp. 1-12 (1983)).

[0041] As used herein, "isolated polypeptide" or "purified polypeptide" is meant to mean a polypeptide (or a fragment thereof) that is substantially free from the materials with which the polypeptide is normally associated in nature. The polypeptides of the invention, or fragments thereof, can be obtained, for example, by extraction from a natural source (for example, a mammalian cell), by expression of a recombinant nucleic acid encoding the polypeptide (for example, in a cell or in a cell-free translation system), or by chemically synthesizing the polypeptide. In addition, polypeptide fragments may be obtained by any of these methods, or by cleaving full length proteins and/or polypeptides.

[0042] As used herein, "peptidomimetic" means a mimetic of a function of a protein which includes some alteration of the normal peptide chemistry. Peptidomimetics typically

are short sequences of amino acids that in biological properties, mimic the function(s) of a particular protein. Peptide analogs enhance some property of the original peptide, such as increase stability, increased efficacy, enhanced delivery, increased half life, etc. Methods of making peptidomimetics based upon a known polypeptide sequence is described, for example, in U.S. Patent Nos. 5,631,280; 5,612,895; and 5,579,250. Use of peptidomimetics can involve the incorporation of a non-amino acid residue with non-amide linkages at a given position. One embodiment of the present invention is a peptidomimetic wherein the compound has a bond, a peptide backbone or an amino acid component replaced with a suitable mimic. Some non-limiting examples of unnatural L- or D-amino acids which may be suitable amino acid mimics include β-alanine, L-α-amino butyric acid, L-γ-amino butyric acid, L-α-amino isobutyric acid, L-ε-amino caproic acid, 7-amino heptanoic acid, L-aspartic acid, L-glutamic acid, N-ε-Boc-N-α-CBZ-L-lysine, N-ε-Boc-N-α-Fmoc-L-lysine, L-methionine sulfone, L-norleucine, L-norvaline, N-α-Boc-N-δCBZ-L-ornithine, N-δ-Boc-N-α-CBZ-L-ornithine, Boc-p-nitro-L-phenylalanine, Boc-hydroxyproline, and Boc-L-thioproline.

[0043] The word "or" as used herein means any one member of a particular list and also includes any combination of members of that list.

[0044] The phrase "nucleic acid" as used herein refers to a naturally occurring or synthetic oligonucleotide or polynucleotide, whether DNA or RNA or DNA-RNA hybrid, single-stranded or double-stranded, sense or antisense, which is capable of hybridization to a complementary nucleic acid by Watson-Crick base-pairing. Nucleic acids of the invention can also include nucleotide analogs (e.g., BrdU), and non-phosphodiester internucleoside linkages (e.g., peptide nucleic acid (PNA) or thiodiester linkages). In particular, nucleic acids can include, without limitation, DNA, RNA, cDNA, gDNA, ssDNA, dsDNA or any combination thereof

[0045] As used herein, "isolated nucleic acid" or "purified nucleic acid" is meant to mean DNA that is free of the genes that, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, such as an autonomously replicating plasmid or virus; or incorporated into the genomic DNA of a prokaryote or eukaryote (e.g., a transgene); or which exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR, restriction endonuclease digestion, or chemical or in vitro synthesis). It also includes a recombinant

DNA which is part of a hybrid gene encoding additional polypeptide sequence. The term "isolated nucleic acid" also refers to RNA, e.g., an mRNA molecule that is encoded by an isolated DNA molecule, or that is chemically synthesized, or that is separated or substantially free from at least some cellular components, for example, other types of RNA molecules or polypeptide molecules.

[0046] As used herein, "sample" is meant to mean an animal; a tissue or organ from an animal; a cell (either within a subject, taken directly from a subject, or a cell maintained in culture or from a cultured cell line); a cell lysate (or lysate fraction) or cell extract; or a solution containing one or more molecules derived from a cell or cellular material (e.g. a polypeptide or nucleic acid), which is assayed as described herein. A sample may also be any body fluid or excretion (for example, but not limited to, blood, urine, stool, saliva, tears, bile) that contains cells or cell components.

[0047] As used herein, "modulate" is meant to mean to alter, by increasing or decreasing.

[0048] As used herein, "effective amount" of a compound is meant to mean a sufficient amount of the compound to provide the desired effect. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of disease (or underlying genetic defect) that is being treated, the particular compound used, its mode of administration, and the like. Thus, it is not possible to specify an exact "effective amount." However, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

[0049] As used herein, "prevent" is meant to mean minimize the chance that a subject who has an increased susceptibility for developing *A. platys* infection will develop *A. platys* infection.

[0050] As used herein, "specifically binds" is meant that an antibody recognizes and physically interacts with its cognate antigen (for example, the disclosed *A. platys* peptides) and does not significantly recognize and interact with other antigens; such an antibody may be a polyclonal antibody or a monoclonal antibody, which are generated by techniques that are well known in the art.

[0051] As used herein, "probe," "primer," or oligonucleotide is meant to mean a single-stranded DNA or RNA molecule of defined sequence that can base-pair to a second DNA or

RNA molecule that contains a complementary sequence (the "target"). The stability of the resulting hybrid depends upon the extent of the base-pairing that occurs. The extent of base-pairing is affected by parameters such as the degree of complementarity between the probe and target molecules and the degree of stringency of the hybridization conditions. The degree of hybridization stringency is affected by parameters such as temperature, salt concentration, and the concentration of organic molecules such as formamide, and is determined by methods known to one skilled in the art. Probes or primers specific for nucleic acids capable of encoding the disclosed *A. platys* peptides (for example, genes and/or mRNAs) have at least 80% - 90% sequence complementarity, preferably at least 91% - 95% sequence complementarity, more preferably at least 96% - 99% sequence complementarity, and most preferably 100% sequence complementarity to the region of the nucleic acid capable of encoding the disclosed *A. platys* peptides to which they hybridize. Probes, primers, and oligonucleotides may be detectably-labeled, either radioactively, or non-radioactively, by methods well-known to those skilled in the art. Probes, primers, and oligonucleotides are used for methods involving nucleic acid hybridization, such as: nucleic acid sequencing, reverse transcription and/or nucleic acid amplification by the polymerase chain reaction, single stranded conformational polymorphism (SSCP) analysis, restriction fragment polymorphism (RFLP) analysis, Southern hybridization, Northern hybridization, in situ hybridization, and electrophoretic mobility shift assay (EMSA).

[0052] As used herein, "specifically hybridizes" is meant to mean that a probe, primer, or oligonucleotide recognizes and physically interacts (that is, base-pairs) with a substantially complementary nucleic acid (for example, a nucleic acid capable of encoding the disclosed *A. platys* peptides) under high stringency conditions, and does not substantially base pair with other nucleic acids.

[0053] As used herein, "high stringency conditions" is meant to mean conditions that allow hybridization comparable with that resulting from the use of a DNA probe of at least 40 nucleotides in length, in a buffer containing 0.5 M NaHPO4, pH 7.2, 7% SDS, 1 mM EDTA, and 1% BSA (Fraction V), at a temperature of 65°C, or a buffer containing 48% formamide, 4.8X SSC, 0.2 M Tris-Cl, pH 7.6, 1X Denhardt's solution, 10% dextran sulfate, and 0.1% SDS, at a temperature of 42°C. Other conditions for high stringency hybridization, such as for PCR, Northern, Southern, or *in situ* hybridization, DNA sequencing, etc., are well-known

by those skilled in the art of molecular biology. (See, for example, F. Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, 1998).

[0054] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Every numerical range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

[0055] In addition, where features or aspects of the inventions are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

## COMPOSITIONS

[0056] Described herein are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

[0057] Also disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, *etc.* of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein.

[0058] Described herein are compositions and methods for the detection of *Anaplasma platys* in a sample obtained from an animal, particularly a member of the *Canidae* family. One embodiment of the invention provides a PCR-based method for the amplification of minute amounts of *A. platys* DNA isolated from canines. For example, and not to be limiting, amplification of DNA can be carried out with a high fidelity Taq polymerase.

***Polynucleotides***

[0059] Described herein are isolated or purified nucleotides. For example, disclosed herein are *Anaplasma platys* nucleotides. The disclosed *Anaplasma platys* nucleotides can be used in one or more of the methods disclosed herein.

[0060] As used herein, "*Anaplasma platys* nucleotides" or "*Anaplasma platy* polynucleotides" refers to the P44 or the OMP-1X nucleotide sequences as well as combinations or fragments thereof described herein. For example, *Anaplasma platys* nucleotides include, but are not limited to, the P44 nucleotide sequences provided in the Figures as well as the sequences provided in SEQ ID NOs: 30-38, SEQ ID NOs: 46-51, combinations thereof as well as fragments thereof. Such sequences can also be referred to as *Anaplasma platys* P44 nucleotides or *Anaplasma platys* P44 polynucleotides. Additional examples of *Anaplasma platys* P44 nucleotides include, but are not limited to Genebank Accession Nos: GQ868750, GU357491, GU357492, GU357493, GU357494, GU357495, GU357496, GU357497, and. HQ738571.

[0061] Also disclosed herein are regions of the *Anaplasma platys* P44 and OMP-1X peptides that have been identified as being highly antigenic as identified through the Jameson-Wolf method as well through a surface probability plot analysis. These regions are herein referred to as a "Box" regions. For example, six regions of the *Anaplasma platys* P44

protein sequence have been identified in Figures 19 and 23. These six regions, from the N-terminal to the C- terminal regions are herein referred to as "P44 Box 1", "P44 Box 2", "P44 Box 3", "P44 Box 4", "P44 Box 5", and "P44 Box 6", respectively. In addition, two regions of the *Anaplasma platys* OMP-1X protein sequence have been identified in Figure 22. These two regions, from the N- terminal to the C- terminal regions are herein referred to as "OMP-1X Box 1" and "OMP-1X Box 2", respectively.

[0062] Disclosed herein are isolated or purified polynucleotides that consist of or comprise the nucleotide sequence of "P44 Box 1". P44 Box 1 includes, but is not limited to, SEQ ID NO: 46. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of hybridizing to or amplifying the sequence of "P44 Box 1". For example, P44 Box 1 primers can include, but are not limited to: SEQ ID NOs: 52 and 53.

[0063] Disclosed herein are isolated or purified polynucleotides that consist of or comprise the nucleotide sequence of "P44 Box 2". P44 Box 2 includes, but is not limited to, SEQ ID NO: 47. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of hybridizing to or amplifying the sequence of "P44 Box 2". For example, P44 Box 1 primers can include, but are not limited to: SEQ ID NOs: 54 and 55.

[0064] Disclosed herein are isolated or purified polynucleotides that consist of or comprise the nucleotide sequence of "P44 Box 3". P44 Box 3 includes, but is not limited to, SEQ ID NO: 48. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of hybridizing to or amplifying the sequence of "P44 Box 3". For example, P44 Box 1 primers can include, but are not limited to: SEQ ID NOs: 56 and 57.

[0065] Disclosed herein are isolated or purified polynucleotides that consist of or comprise the nucleotide sequence of "P44 Box 4". P44 Box 4 includes, but is not limited to, SEQ ID NO: 49. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of hybridizing to or amplifying the sequence of "P44 Box 4". For example, P44 Box 1 primers can include, but are not limited to: SEQ ID NOs: 58 and 59.

[0066] Disclosed herein are isolated or purified polynucleotides that consist of or comprise the nucleotide sequence of "P44 Box 5". P44 Box 5 includes, but is not limited to, SEQ ID NO: 50. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of hybridizing to or amplifying the sequence of "P44 Box 5". For example, P44 Box 1 primers can include, but are not limited to: SEQ ID NOs: 60 and 61.

[0067] Disclosed herein are isolated or purified polynucleotides that consist of or comprise the nucleotide sequence of "P44 Box 6". P44 Box 6 includes, but is not limited to, SEQ ID NO: 51. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of hybridizing to or amplifying the sequence of "P44 Box 6". For example, P44 Box 1 primers can include, but are not limited to: SEQ ID NOs: 62 and 63.

[0068] Also disclosed are primers that can be used to amplify one or more *Anaplasma platys* P44 nucleotides. Examples include, but are not limited to SEQ ID NOs: 82-91.

[0069] *Anaplasma platys* nucleotides include, but are not limited to, the OMP-1X nucleotide sequences provided in the Figures as well as the sequences provided in SEQ ID NOs: 11-17, combinations thereof as well as fragments thereof. Such sequences can also be referred to as *Anaplasma platys* OMP-1X nucleotides or *Anaplasma platys* OMP-1X polynucleotides. Other examples of *Anaplasma platys* OMP-1X nucleotides include, but are not limited to the sequences provided in GenBank Accession Nos: GQ868750, HQ738571, GU357491.

[0070] Disclosed herein are isolated or purified polynucleotides that consist of or comprise the nucleotide sequence of "OMP-1X Box 1". OMP-1X Box 1 includes, but is not limited to, SEQ ID NO: 14. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of hybridizing to or amplifying the sequence of "OMP-1X Box 1".

[0071] Disclosed herein are isolated or purified polynucleotides that consist of or comprise the nucleotide sequence of "OMP-1X Box 2". OMP-1X Box 2 includes, but is not limited to, SEQ ID NO: 15. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of hybridizing to or amplifying the sequence of "OMP-1X Box 2".

[0072] The polynucleotides described herein can contain less than an entire microbial genome and can be RNA, DNA, or combinations thereof. Polynucleotides described herein can be isolated. An isolated polynucleotide is a naturally-occurring polynucleotide that is not immediately contiguous with one or both of the 5′ and 3′ flanking genomic sequences that it is naturally associated with. Isolated polynucleotides can also include non-naturally occurring nucleic acid molecules. In some aspects, polynucleotides can also comprise fragments that encode immunogenic polypeptides.

[0073] In some aspects, polynucleotides described herein can be probes or primers, for example, PCR primers, to detect the presence of *A. platys* polynucleotides in a biological sample. Probes are molecules capable of interacting with a target nucleic acid, typically in a sequence specific manner, for example, through hybridization. Primers are a subset of probes that can support an enzymatic manipulation and that can hybridize with a target nucleic acid sequence. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art that do not interfere with the manipulation of peptides, enzymes, or proteins. In one aspect, the primers disclosed herein can comprise any of the isolated polynucleotides described herein. For example, and not to be limiting, the isolated polynucleotide can be SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 99.

[0074] The hybridization of nucleic acids is well understood in the art and hence need not be discussed herein. Typically a primer can be made from any combination of nucleotides, nucleotide derivatives, and analogs available in the art. The ability of such probes and primers to specifically hybridize to *A. platys* polynucleotide sequences can enable the primer to be used for the detection of the presence of complementary sequences. In some embodiments, polynucleotide primers and probes of the invention described herein can hybridize to complementary sequences in a sample, including saliva, blood, plasma, serum, cerebrospinal fluid, and tissue. In some embodiments, the polynucleotides from the sample can be subjected to gel electrophoresis, size separation techniques, immobilization without size separation, and labeling. Suitable labels and methods for labeling primers are known in

the art and include radioactive labels, biotin labels, fluorescent labels, bioluminescent labels, and enzyme labels.

[0075] When referring to a nucleotide sequence "N" represents any of the four common nucleotides (*e.g.*, A, C, G, or T), "M" represents either an A or C nucleotide, "S" will be defined to mean C or G, and "Y" will be defined henceforth as C or T. For example, SEQ ID NO: 85 (GCAAACCTAACACCMAAYTCMCCACC) includes an "M" at positions 15 and 22. As such, position 15 or 22 of SEQ ID NO: 85 can be an A or C nucleotide. In addition, SEQ ID NO: 85 includes a "Y" at position 19. As such, position 9 of SEQ ID NO: 85 can be a C or T.

***Primers***

[0076] Disclosed herein are P44 primer sets comprising F1 through F3 and R1 through R5. Each p44 primer set comprises a first primer, *i.e.*, forward, and a second primer, *i.e.*, reverse, both of which can be about 10 to about 35 nucleotides in length or a primer of alternant length (*e.g.*, 10-15, 10-20, 10-25, 10-30, 15-20, 15-25, 15-30, 15-35, 20-25, 20-30, 20-35, 25-30, 25-35, 30-35). The first primer comprises a sequence that is complementary to a consecutive sequence of at least 10 nucleotides in length, within the following sequences: ATTATGTATGATTTATCCTAAGTTATCTGAG (SEQ ID NO: 82), GGGATATCGGCGTTGATAGGG (SEQ ID NO: 83), and GGTTTGTGTTGCTGGTGATTGGAGG (SEQ ID NO: 84). The second primer comprises a sequence which is complementary to the inverse complement of a consecutive sequence of at least 10 nucleotides in length, within the following sequences: GCAAACCTAACACCMAAYTCMCCACC (SEQ ID NO: 85), TATACTAAAAAAGAATTAAGTCAAGAG (SEQ ID NO: 86), ATGGTAGAAASCCCCAGCAAA (SEQ ID NO: 87), CACGTNTTTAGTTACTGCCA (SEQ ID NO: 88), and GTACTAGTCAGCGCCACTAACATCAA (SEQ ID NO: 89). As used herein, "N" represents any of the four common nucleotides (*e.g.*, A, C, G, or T), "M" represents either an A or C nucleotide, "S" will be defined to mean C or G, and "Y" will be defined henceforth as C or T. Such primers can be useful for detecting the presence of *A. platys* in members of the *Anaplasmataceae* family. HVF and HVR (Table 1) are the *A. platys*-specific primers (Figure 16). Using the nested PCR (genus-specific primer Pair F3 and R1 during the first PCR and species-specific primer pair HVF and HVR in second PCR) sensitive and *A. platys*-specific PCR can be performed. Such primers can also useful for

detecting the presence of *A. platys* DNA in samples obtained from ticks or other invertebrate carriers that feed on the vertebrate hosts.

[0077] Also disclosed herein are primers that can be used to synthesize one or more of the A. platys polypeptides described herein. For example, disclosed herein are primers that can be used to produce an *A. platys* polypeptide comprising a sequence that is capable of encoding a multimeric *A. platys* polypeptide wherein the intervening sequence present in the full *A. platys* P44 or OMP-1X nucleotide or peptide sequence are removed. For example, disclosed herein are primers that can be used to add a restriction site into a nucleic acid sequence described herein through inverse PCR. The nucleotide can then be digested and self-ligated to remove a specific intervening sequence. For example, SEQ ID NO: 66 (OMP-1X box 1 and box 2 Forward primer: AACATATGAATCTTGTGAGCGCGG) can be used to introduce an NdeI site in combination with SEQ ID NO: 67 (OMP-1X box 1 and box 2 Reverse primer: GGGGATCCGGCTGGGGGAGCAGAAG) which can introduce a BamHI site.

[0078] Also disclosed are primers that can be used to remove an intervening sequence between OMP-1X Box 1 and Box 2. For example, the primer pair of SEQ ID NO: 68 can be used in combination with SEQ ID NO: 69.

[0079] Also disclosed are primers that can be used to remove an intervening sequence between P44 Box 1 and Box 2. For example, the primer pair of SEQ ID NO: 70 can be used in combination with SEQ ID NO: 71.

[0080] Also disclosed are primers that can be used to remove the intervening sequence of P44 Box 1 and Box 2. For example, the primer pair of SEQ ID NO: 72 can be used in combination with SEQ ID NO: 73.

[0081] Also disclosed are primers that can be used to remove the intervening sequence of P44 Box 3 and Box 4. For example, the primer pair of SEQ ID NO: 74 can be used in combination with SEQ ID NO: 75 or SEQ ID NO: 76 can be used in combination with SEQ ID NO: 77

[0082] Also disclosed are primers that can be used to remove the intervening sequence of P44 Box 5 and Box 6. For example, the primer pair of SEQ ID NO: 78 can be used in

combination with SEQ ID NO: 79 or SEQ ID NO: 80 can be used in combination with SEQ ID NO: 81

[0083] Also disclosed are methods for detecting *A. platys* provides a p44 primer set comprising a first primer sequence which can be complementary to a sequence of the *A. platys* p44 gene sense strand and a second primer which can be complementary to the sequence of the *A.* platys p44 gene antisense strand, amplifying the DNA in the sample using a polymerase chain reaction (PCR) and the p44 primer set, and determining the length which corresponds to the sequence or length of that portion to which the first p44 primer and the second p44 primer bind is indicative of the presence of *A. platys* in the DNA sample.

[0084] Also disclosed herein are aspects related to primers in the p44 primer set. The first p44 and the second p44 primers can be from about 10 to about 35 nucleotides in length or a primer of alternant length (*e.g.*, 10-15, 10-20, 10-25, 10-30, 15-20, 15-25, 15-30, 15-35, 20-25, 20-30, 20-35, 25-30, 25-35, 30-35). The first p44 primer, comprises a sequence which is substantially identical to the complement of a consecutive sequence of at least 10 nucleotides in length, within the following sequence: GAAGAATACGAAAGCGGCGG (SEQ ID NO: 90). In some embodiments, the primer can be capable of hybridizing to a target sequence.

[0085] The second primer comprises a sequence which can be complementary to the inverse complement of a consecutive sequence of at least 10 nucleotides in length, within the following sequence: TACTTAGGTCTTCCGCTTTCGC (SEQ ID NO: 91).

[0086] HVF and HVR (Table 1 and Figure 16) can be useful for detecting the presence of *A. platys* in samples obtained from vertebrate animals such as humans or dogs, or from the invertebrate vectors such as brown ticks, which can transmit this pathogen from one vertebrate animal to another.

[0087] Also disclosed herein are compositions and methods for detecting the presence of *A. platys* in samples obtained from a vertebrate or invertebrate animal. The method comprises amplifying the DNA contained within the sample using a primer set comprising primers which comprise sequences that can be complementary to select regions of the *p44* gene of *A. platys* and a polymerase chain reaction (PCR) to provide a pool of PCR products, and then assaying the pool for the presence or absence of a PCR product whose length or sequence indicates that PCR product corresponds to the region of the *p44* gene that is flanked

by the nucleotide sequences which are complementary to the first and second members of the p44 primer set. The tools are the members of the p44 primer sets. Multiple *A. platys p44* gene sequences are set forth in GenBank under accession No. GQ868750 and GU357491, respectively. Additional *p44ES* and *p44* sequences were set forth in GenBank under accession No. GU357492, GU357493, GU357494, GU357495, GU357496 and GU357497.

[0088] In some embodiments, the primers in the p44 primer set can be based upon select sequences in the p44 gene of *A. platys*. The *p44* gene encodes a major outer membrane protein of *A. platys*. The sequences of the first and second primers in the p44 primer set are distinct from sequences found in the closely related *p44* gene homologs in *A. phagocytophilum* or *A. marginale*. The first primer in the p44 primer set can be an oligonucleotide of various lengths, including but not limited to 10-15, 10-20, 10-25, 10-30, 15-20, 15-25, 15-30, 15-35, 20-25, 20-30, 20-35, 25-30, 25-35, 30-35, and 10 to 35 nucleotides in length. In some embodiments, the first primer can be at least 10 nucleotides in length. The second p44 primer in the *A. platys* primer set can be an oligonucleotide of 10-15, 10-20, 10-25, 10-30, 15-20, 15-25, 15-30, 15-35, 20-25, 20-30, 20-35, 25-30, 25-35, 30-35, or 10 to 35 nucleotides in length. In one embodiment, the second p44 primer can be at least about 10 nucleotides in length. The first p44 primer can comprise a sequence which is substantially identical to the complement of consecutive sequence located between nucleotide positions 540-559 of the sense strand of the open reading frame sequence of the *p44* gene of *A. platys*.

[0089] As used herein the term "substantially identical" means that the sequence is at least 90% identical, at least 95% identical, or 100% identical to a particular reference sequence (nucleotides 540-559 or 812-849) within Figure 22.

[0090] The second p44 primer, comprises a sequence which is substantially identical to and the inverse of a consecutive sequence located between nucleotides 812-849 of the sense strand of the *p44* gene of *A. platys*. The sequence of the second p44 primer is substantially identical to the complement of the inverse complement of a consecutive sequence contained within Figure 22. In some embodiments, the primers can be capable of hybridizing to target sequences.

[0091] In specific embodiments, the first and second primers in the p44 primer set can comprise the sequences shown in Table 1. The first and second primers can also comprise

sequences which are shorter by one to ten nucleotides than the sequences shown in Table 1 below. The first and second primers of the *A. platys* primer set can also comprise a sequence which is longer than the sequences shown in Table 1 below. Such sequences can have one to ten additional nucleotides attached to the 5' end of the above-listed sequences. The additional nucleotides can be selected from the group consisting of adenine, cytosine, guanine, thymine, adenylic acid, guanylic acid, and combinations thereof.

[0092] In another embodiment, the sequence of the nine first and second p44 primer sets shown in Table 1 can be based upon a comparison of the open-reading frame sequences of nine *A. platys* isolates. Such primer sets can specifically amplify the target sequence of multiple *A. platys* isolates, but not *A. phagocytophilum* or *A. marginale* isolates. The primers shown in Table 1 are both species-universal (*e.g.*, F1, F2, F3, R1, R2, R3, R4, and R5) and species-specific (*e.g.*, HVF and HVR) for *A. platys*.

[0093] Disclosed herein are isolated polynucleotides encoding an outer membrane protein of *Anaplasma platys*, or a fragment thereof. In one aspect, the outer membrane protein can be P44 or OMP-1X protein. In a further aspect, disclosed herein are isolated polynucleotides comprising any of the sequences described herein, or a fragment thereof. For example, and not to be limiting, the polynucleotide sequence can be SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or a fragment thereof. In yet a further aspect, the isolated polynucleotide sequence can be a polynucleotide capable of encoding any peptide sequence described herein or a fragment thereof. For example and not to be limiting, the polynucleotide sequence can be a polynucleotide capable of encoding SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, or a fragment thereof. By way of further example, the polynucleotide sequences disclosed herein can also be polynucleotides capable of encoding the amino acid sequence comprising the amino acid from about position

20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, a combination or a fragment thereof. In still a further aspect, disclosed herein are isolated polynucleotides that encode the peptide sequences described herein. For example, and not to be limiting, the polynucleotide sequence can encode P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, OMP-1X Box 1, OMP-1X Box 2, OMP-1X Box 1 and OMP-1X Box 2, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof. By way of further example, the polynucleotide sequences disclosed herein can also be polynucleotides that can encode the amino acid sequence comprising the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof.

[0094] Also disclosed herein are isolated polynucleotides that encode variants of the proteins described herein. In one aspect, disclosed herein are isolated polynucleotides that can encode a variant of an outer membrane protein of *Anaplasma platys*, or a fragment thereof. For example, and not to be limiting, the outer membrane protein can be P44 or OMP-1X protein. In a further aspect, the polynucleotide can encode a variant that can have at least 95% identity to, for example, and not to be limiting, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, the amino acid from about

position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof. In a further aspect, the variant can be immunoreactive with at least one antibody that binds to P44 protein, P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, OMP-1X protein, OMP-1X Box 1, OMP-1X Box 2, OMP-1X Box 1 and OMP-1X Box 2, or a fragment thereof.

[0095] Disclosed herein are polynucleotides that contain less than an entire microbial genome and can be single- or double-stranded nucleic acids. A polynucleotide can be RNA, DNA, cDNA, genomic DNA, chemically synthesized RNA or DNA or combinations thereof. The polynucleotides can be purified free of other components, such as proteins, lipids and other polynucleotides. For example, the polynucleotide can be 50%, 75%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% purified. The polynucleotides described herein can encode one or more of the polypeptides described elsewhere herein. For example, disclosed herein are polynucleotides capable of encoding the peptides described herein, for example: an *Anaplasma platys* P44 or OMP-1X protein; a variant of the *Anaplasma platys* P44 or OMP-1X protein; or an antigenic fragment of the *Anaplasma platys* P44 or OMP-1X protein, or fragments thereof. Polynucleotides can comprise other nucleotide sequences, such as sequences coding for linkers, signal sequences, TMR stop transfer sequences, transmembrane domains, or ligands useful in protein purification such as glutathione-S-transferase, histidine tag, and Staphylococcal protein A.

[0096] The polynucleotides disclosed herein can be isolated. An isolated polynucleotide is a naturally-occurring polynucleotide that is not immediately contiguous with one or both of the 5' and 3' flanking genomic sequences that it is naturally associated with. An isolated polynucleotide can be, for example, a recombinant DNA molecule of any length, provided that the nucleic acid sequences naturally found immediately flanking the recombinant DNA molecule in a naturally-occurring genome is removed or absent. Isolated polynucleotides can also include non-naturally occurring nucleic acid molecules. A nucleic acid molecule existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or

genomic libraries, or gel slices containing a genomic DNA restriction digest are not to be considered an isolated polynucleotide.

[0097] The polynucleotides disclosed herein can also comprise fragments that encode immunogenic polypeptides. The polynucleotides disclosed herein can encode full-length polypeptides, polypeptide fragments, and variant or fusion polypeptides.

[0098] The polynucleotides disclosed herein can be degenerate nucleotide sequences encoding one or more of the polypeptides disclosed herein, as well as homologous nucleotide sequences that are at least about 80, 85, 90, 95, 96, 97, 98, 99% or 100% identical to the polynucleotide sequences disclosed herein and the complements thereof are also disclosed herein. Percent sequence identity can be calculated as described elsewhere herein. Degenerate nucleotide sequences are polynucleotides that encode a polypeptide of the invention or fragments thereof, but differ in nucleic acid sequence from the wild-type polynucleotide sequence, due to the degeneracy of the genetic code. Complementary DNA (cDNA) molecules, species homologs, and variants of *Anaplasma platys* polynucleotides that encode biologically functional *Anaplasma platys* polypeptides also are *Anaplasma platys* polynucleotides.

[0099] The polynucleotides described herein can be isolated from nucleic acid sequences present in, for example, a biological sample, such as blood, serum, saliva, or tissue from an infected individual. Polynucleotides can also be synthesized in the laboratory, for example, using an automatic synthesizer. An amplification method such as PCR can be used to amplify polynucleotides from either genomic DNA or cDNA encoding the polypeptides.

[00100] The polynucleotides disclosed herein can be used, for example, as probes or primers, for example, PCR primers, to detect the presence of *Anaplasma platys* polynucleotides in a test sample, such as a biological sample. Probes are molecules capable of interacting with a target nucleic acid, typically in a sequence specific manner, for example, through hybridization. Primers are a subset of probes that can support an enzymatic manipulation and that can hybridize with a target nucleic acid such that the enzymatic manipulation occurs. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art that do not interfere with the enzymatic manipulation.

[00101] The hybridization of nucleic acids is well understood in the art and discussed herein. Typically a probe can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art. The ability of such probes and primers to specifically hybridize to *Anaplasma platys* P44 or *Anaplasma platys* OMP-1x polynucleotide sequences will enable them to be of use in detecting the presence of complementary sequences in a given test sample. Polynucleotide probes and primers can hybridize to complementary sequences in a test sample such as a biological sample, including, but not limited to, saliva, sputum, blood, plasma, serum, urine, feces, cerebrospinal fluid, amniotic fluid, wound exudate, or tissue. Polynucleotides from the sample can be, for example, subjected to gel electrophoresis or other size separation techniques or can be immobilized without size separation. The polynucleotide probes or primers can be labeled. Suitable labels and methods for labeling probes and primers are known in the art, and include, for example, radioactive labels incorporated by nick translation or by kinase, biotin labels, fluorescent labels, chemiluminescent labels, bioluminescent labels, metal chelator labels and enzyme labels. The polynucleotides from the sample are contacted with the probes or primers under hybridization conditions of suitable stringencies.

[00102] Depending on the application, varying conditions of hybridization can be used to achieve varying degrees of selectivity of the probe or primer towards the target sequence. For applications requiring high selectivity, relatively stringent conditions can be used, such as low salt and/or high temperature conditions, such as provided by a salt concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. For applications requiring less selectivity, less stringent hybridization conditions can be used. For example, salt conditions from about 0.14 M to about 0.9M salt, at temperatures ranging from about 20°C to about 55°C. The presence of a hybridized complex comprising the probe or primer and a complementary polynucleotide from the test sample indicates the presence of *Anaplasma platys* or an *Anaplasma platys* polynucleotide sequence in the sample.

***Polypeptides***

[00103] Described herein are isolated or purified polypeptides. For example, disclosed herein are isolated or purified *Anaplasma platys* polypeptides. The disclosed isolated or purified *Anaplasma platys* polypeptides can be used in one or more of the methods disclosed herein.

[00104] A polypeptide can be a polymer of three or more amino acids covalently linked by amide bonds. A polypeptide can be post-translationally modified. A purified polypeptide can be a polypeptide preparation that is substantially free of cellular material, other peptides and polypeptides, chemical precursors, synthetic chemicals, or combinations thereof.

[00105] As used herein, "*Anaplasma platys* peptides" or "*Anaplasma platy* proteins" refers to the P44 or the OMP-1X peptide sequences as well as combinations or fragments thereof described herein. For example, *Anaplasma platys* peptides include, but are not limited to, the P44 amino acid sequences provided in the Figures as well as the sequences provided in SEQ ID NOs: 21-29, SEQ ID NOs: 39-45, SEQ ID NOs: 92-98, combinations thereof as well as fragments thereof. Such sequences can also be referred to as *Anaplasma platys* P44 peptides or *Anaplasma platys* P44 proteins. Other examples of *Anaplasma platys* P44 peptides include, but are not limited to the sequences provided in GenBank Accession Nos: GQ868750, GU357491, GU357492, GU357493, HQ738571, GU357494, GU357495, GU357496, and GU357497.

[00106] *Anaplasma platys* peptides also include, but are not limited to, the OMP-1X amino acid sequences provided in the Figures as well as the sequences provided in SEQ ID NOs: 1-11, combinations thereof as well as fragments thereof. Such sequences can also be referred to as *Anaplasma platys* OMP-1X proteins. Other examples of *Anaplasma platys* OMP-1X peptides include, but are not limited to the sequences provided in GenBank Accession Nos: GQ868750, HQ738571, and GU357491.

[00107] Also disclosed herein are regions of the *Anaplasma platys* P44 and OMP-1X peptides that have been identified as being highly antigenic as identified through the Jameson-Wolf method as well through a surface probability plot analysis. These regions are herein referred to as a "Box" regions. For example, six regions of the *Anaplasma platys* P44 protein sequence have been identified in Figures 19 and 23. These six regions, from the N-terminal to the C- terminal regions are herein referred to as "P44 Box 1", "P44 Box 2", "P44 Box 3", "P44 Box 4", "P44 Box 5", and "P44 Box 6", respectively. In addition, two regions of the *Anaplasma platys* OMP-1X protein sequence have been identified in Figure 22. These two regions, from the N- terminal to the C- terminal regions are herein referred to as "OMP-1X Box 1" and "OMP-1X Box 2", respectively.

[00108] Disclosed herein are isolated or purified polypeptides that consist of or comprise the amino acid sequence of "P44 Box 1". P44 Box 1 includes, but is not limited to, the P44 Box 1 amino acid sequences identified in Figures 19 or 23, for example the sequence from about position 22 to about position 39 of Figure 19 or the sequence from about position 40 to about position 64 of Figure 23. In addition, P44 Box 1 includes, but is not limited to, the amino acid sequences of SEQ ID NO: 39, SEQ ID NO: 92, SEQ ID NOs: 100-103, the amino acid sequences from about position 22 to about position 39 of SEQ ID NOs: 21-24, or the amino acid sequence encoded by SEQ ID NO: 46. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of encoding the amino acid sequence of "P44 Box 1".

[00109] Disclosed herein are isolated or purified polypeptides that consist of or comprise the amino acid sequence of "P44 Box 2". P44 Box 2 includes, but is not limited to, the P44 Box 2 amino acid sequences identified in Figures 19 or 23, for example the sequence from about position 77 to about position 85 of Figure 19 or the sequence from about position 102 to about position 111 of Figure 23. In addition, P44 Box 2 includes, but is not limited to, the amino acid sequences of SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NOs: 104-107, the amino acid sequences from about position 77 to about position 85 of SEQ ID NOs: 21-24, or the amino acid sequence encoded by SEQ ID NO: 47. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of encoding the amino acid sequence of "P44 Box 2".

[00110] Disclosed herein are isolated or purified polypeptides that consist of or comprise the amino acid sequence of "P44 Box 3". P44 Box 3 includes, but is not limited to, the P44 Box 3 amino acid sequences identified in Figures 19 or 23, for example the sequence from about position 151 to about position 190 of Figure 19 or the sequence from about position 178 to about position 222 of Figure 23. In addition, P44 Box 3 includes, but is not limited to, the amino acid sequences of SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NOs: 108-111, the amino acid sequences from about position 151 to about position 191 of SEQ ID NOs: 21-24, or the amino acid sequence encoded by SEQ ID NO: 48. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of encoding the amino acid sequence of "P44 Box 3".

[00111] Disclosed herein are isolated or purified polypeptides that consist of or comprise the amino acid sequence of "P44 Box 4". P44 Box 4 includes, but is not limited to, the P44

Box 4 amino acid sequences identified in Figures 19 or 23, for example the sequence from about position 227 to about position 234 of Figure 19 or the sequence from about position 259 to about position 266 of Figure 23. In addition, P44 Box 4 includes, but is not limited to, the amino acid sequences of SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NOs: 112-115, the amino acid sequences from about position 207 to about position 214 of SEQ ID NOs: 21-24, or the amino acid sequence encoded by SEQ ID NO: 49. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of encoding the amino acid sequence of "P44 Box 4".

[00112] Disclosed herein are isolated or purified polypeptides that consist of or comprise the amino acid sequence of "P44 Box 5". P44 Box 5 includes, but is not limited to, the P44 Box 5 amino acid sequences identified in Figures 19 or 23, for example the sequence from about position 276 to about position 308 of Figure 19 or the sequence from about position 319 to about position 340 of Figure 23. In addition, P44 Box 5 includes, but is not limited to, the amino acid sequences of SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NOs: 116-119, the amino acid sequences from about position 248 to about position 269 of SEQ ID NOs: 21-24, or the amino acid sequence encoded by SEQ ID NO: 50. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of encoding the amino acid sequence of "P44 Box 5".

[00113] Disclosed herein are isolated or purified polypeptides that consist of or comprise the amino acid sequence of "P44 Box 6". P44 Box 6 includes, but is not limited to, the P44 Box 6 amino acid sequences identified in Figures 19 or 23, for example the sequence from about position 417 to about position 426 of Figure 19 or the sequence from about position 451 to about position 460 of Figure 23. In addition, P44 Box 6 includes, but is not limited to, the amino acid sequences of SEQ ID NO: 44, SEQ ID NO: 97, SEQ ID NOs: 120-123, the amino acid sequences from about position 378 to about position 386 of SEQ ID NOs: 21-24, or the amino acid sequence encoded by SEQ ID NO: 51. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of encoding the amino acid sequence of "P44 Box 6".

[00114] Disclosed herein are isolated or purified polypeptides that consist of or comprise the amino acid sequence of "OMP-1X Box 1". OMP-1X Box 1 includes, but is not limited to, the OMP-1X Box 1 amino acid sequences identified in Figure 22, for example the sequence from about position 66 to about position 192 of Figure 22. In addition, OMP-1X

Box 1 includes, but is not limited to, the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 6, or SEQ ID NO: 14. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of encoding the amino acid sequence of "OMP-1X Box 1".

[00115] Disclosed herein are isolated or purified polypeptides that consist of or comprise the amino acid sequence of "OMP-1X Box 2". OMP-1X Box 2 includes, but is not limited to, the OMP-1X Box 2 amino acid sequences identified in Figure 22, for example the sequence from about position 241 to about position 309 of Figure 22. In addition, OMP-1X Box 2 includes, but is not limited to, the amino acid sequences of SEQ ID NO: 3, SEQ ID NO: 7, or SEQ ID NO: 15. Also disclosed herein are isolated or purified polynucleotides that consist of or comprise a polynucleotide sequence capable of encoding the amino acid sequence of "OMP-1X Box 2".

[00116] Also described herein are purified polypeptides comprising the sequences outlined in Figure 17, or at least about 10 contiguous amino acids of the sequence from Figure 17 wherein the at least 10 contiguous amino acids are chosen from amino acids 300-410. In some aspects, the polypeptides described herein also inherently disclose the nucleotide sequence as related to the amino acids sequence 300-410 from Figure 17.

[00117] In one aspect, described herein are purified polypeptides comprising at least about 8, 10, 15, 20, 30, 40, 50, or more contiguous amino acids, wherein the contiguous amino acids can be chosen from amino acids 300-410 from Figure 17.

[00118] Disclosed herein are purified polypeptides that can either be full-length polypeptides or fragments of polypeptides. For example, fragments of polypeptides disclosed herein can comprise about 8, 10, 15, 20, 30, 40, 50, or more amino acids of polypeptides of the aspects described herein. Variant polypeptides can be at least about 90, 96, 98, or 99% identical to the polypeptide sequences shown in Figures 17 and 22. Variant polypeptides can have one or more conservative amino acid variations or other minor modifications and retain biological activity, *i.e.*, are biologically functional equivalents. A biologically active equivalent can have substantially equivalent function when compared to the corresponding wild-type polypeptide.

[00119] Described herein are isolated or purified polypeptides comprising a sequence chosen from the following: a *Anaplasma platys* P44 protein, a variant of an *Anaplasma platys*

P44 protein, or an antigenic fragment of an *Anaplasma platys* P44 protein. In one aspect, the *Anaplasma platys* P44 protein can comprise or consist of: P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof.

[00120] In a further aspect, the *Anaplasma platys* P44 protein can comprise or consist of a variant of: P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO:

94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof.

[00121] It is understood that one way to define the variants and derivatives of the disclosed proteins herein is to define them in terms of homology/identity to specific known sequences. Specifically disclosed are variants of *A. platys* peptides and other proteins or peptides herein disclosed which have at least, 70% or at least 75% or at least 80% or at least 85% or at least 90% or at least 95% homology to the *A. platys* peptides specifically recited herein. Those of skill in the art readily understand how to determine the homology of two proteins.

[00122] As this specification discusses various polypeptides and polypeptide sequences it is understood that the nucleic acids that can encode those polypeptide sequences are also disclosed. This would include all degenerate sequences related to a specific polypeptide sequence, i.e. all nucleic acids having a sequence that encodes one particular polypeptide sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the

disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed polypeptide sequences.

[00123] In yet a further aspect, the *Anaplasma platys* P44 protein can comprise or consist of an antigenic fragment of: P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, or the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19. In still a further aspect, the *Anaplasma platys* P44 proteins described herein can comprise or consist of a combination of one or more of the sequences described herein.

[00124] In one aspect, the variants or antigenic fragments of the *Anaplasma platys* P44 proteins described herein can be immunoreactive with at least one antibody that binds to their corresponding peptide sequence.

[00125] Also described herein are isolated or purified polypeptides that can comprise a sequence that is at least 95% identical to an *Anaplasma platys* P44 protein, a variant of the *Anaplasma platys* P44 protein, or an antigenic fragment of the *Anaplasma platys* P44 protein. Thus, in one aspect, the polypeptides described herein can be at least 95% identical to: P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof.

[00126] In a further aspect, the polypeptides described herein can be at least 95% identical to a variant of: P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof.

[00127] In yet a further aspect, the polypeptides described herein can be at least 95% identical to an antigenic fragment of: P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ

ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, or the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19. In still a further aspect, the polypeptides described herein can be at least 95% identical to one or more of the peptide sequences described herein. In still a further aspect, the *Anaplasma platys* P44 proteins described herein can comprise or consist of a combination of one or more of the sequences described herein.

[00128] In one aspect, the variants or antigenic fragments can be at least 95% identical to the *Anaplasma platys* P44 proteins described herein can be immunoreactive with at least one antibody that binds to their corresponding peptide sequence.

[00129] In one aspect, the isolated polypeptides described herein can be: the P44 Box 1 protein, the P44 Box 2 protein, the P44 Box 3 protein, the P44 Box 4 protein, the P44 Box 5 protein, the P44 Box 6 protein; a variant of the P44 Box 1 protein, the P44 Box 2 protein, the P44 Box 3 protein, the P44 Box 4 protein, the P44 Box 5 protein, the P44 Box 6 protein; or

an antigenic fragment of the P44 Box 1 protein, the P44 Box 2 protein, the P44 Box 3 protein, the P44 Box 4 protein, the P44 Box 5 protein, the P44 Box 6 protein.

[00130] Furthermore, described herein are isolated or purified polypeptides comprising a sequence chosen from the following: a *Anaplasma platys* OMP-1X protein, a variant of the *Anaplasma platys* OMP-1X protein, or an antigenic fragment of the *Anaplasma platys* OMP-1X protein. In one aspect, the *Anaplasma platys* OMP-1X protein can comprise or consist of: OMP-1X Box 1, OMP-1X Box 2, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof.

[00131] In a further aspect, the *Anaplasma platys* OMP-1X protein can comprise or consist of a variant of: OMP-1X Box 1, OMP-1X Box 2, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof.

[00132] In yet a further aspect, the *Anaplasma platys* OMP-1X protein can comprise or consist of an antigenic fragment of: OMP-1X Box 1, OMP-1X Box 2, SEQ ID NO: 1, SEQ

ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, or a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22. In still a further aspect, the *Anaplasma platys* OMP-1X proteins described herein can comprise or consist of a combination of one or more of the sequences described herein.

[00133] In one aspect, the variants or antigenic fragments of the *Anaplasma platys* OMP-1X proteins described herein can be immunoreactive with at least one antibody that binds to their corresponding peptide sequence.

[00134] Also described herein are isolated or purified polypeptides that can comprise a sequence that is at least 95% identical to an *Anaplasma platys* OMP-1X protein, a variant of the *Anaplasma platys* OMP-1X protein, or an antigenic fragment of the *Anaplasma platys* OMP-1X protein. Thus, in one aspect, the polypeptides described herein can be at least 95% identical to: OMP-1X Box 1, OMP-1X Box 2, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof.

[00135] In a further aspect, the polypeptides described herein can be at least 95% identical to a variant of: OMP-1X Box 1, OMP-1X Box 2, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof.

[00136] In yet a further aspect, the polypeptides described herein can be at least 95% identical to an antigenic fragment of: OMP-1X Box 1, OMP-1X Box 2, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, or a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22. In still a further aspect, the *Anaplasma platys* OMP-1X proteins described herein can comprise or consist of a combination of one or more of the sequences described herein. In still a further aspect, the polypeptides described herein can be at least 95% identical to one or more of the peptide sequences described herein. In still a further aspect, the *Anaplasma platys* OMP-1X proteins described herein can comprise or consist of a combination of one or more of the sequences described herein.

[00137] In one aspect, the variants or antigenic fragments can be at least 95% identical to the *Anaplasma platys* OMP-1X proteins described herein can be immunoreactive with at least one antibody that binds to their corresponding peptide sequence.

[00138] In one aspect, the isolated polypeptides described herein can be: the OMP-1X protein, the OMP-1X Box 1 protein, or the OMP-1X Box 2 protein; a variant of the OMP-1X protein, the OMP-1X Box 1 protein, or the OMP-1X Box 2 protein; or an antigenic fragment of the OMP-1X protein, the OMP-1X Box 1 protein, or the OMP-1X Box 2 protein.

[00139] Also disclosed herein are isolated polynucleotides that encode the polypeptides described herein. A purified polypeptide can further comprising a carrier. A purified polypeptide can be in a multimeric form. A purified polypeptide can be linked to an indicator reagent, an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, a transmembrane domain, a protein purification ligand, a heterologous polypeptide or a combination thereof.

[00140] Purified polypeptides described herein can either be full-length polypeptides or fragments of polypeptides. For example, fragments of polypeptides described herein can comprise about 10, 15, 20, 50, 75, 100, 150, 200, 250 or more amino acids of polypeptides of the invention. For example, and not to be limiting, variant polypeptides can be at least about 80, or about 90, 96, 98, or 99% identical to the polypeptide sequences shown in SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof, and are also polypeptides of the invention. Variant polypeptides have one or more conservative amino acid variations or other minor modifications and retain biological activity, i.e., are biologically functional equivalents. A biologically active equivalent has substantially equivalent function when compared to the corresponding wild-type polypeptide.

[00141] Percent sequence identity has an art recognized meaning and there are a number of methods to measure identity between two polypeptide or polynucleotide sequences. See, e.g., Lesk, Ed., Computational Molecular Biology, Oxford University Press, New York, (1988); Smith, Ed., Biocomputing: Informatics And Genome Projects, Academic Press, New York,

(1993); Griffin & Griffin, Eds., Computer Analysis Of Sequence Data, Part I, Humana Press, New Jersey, (1994); von Heinje, Sequence Analysis In Molecular Biology, Academic Press, (1987); and Gribskov & Devereux, Eds., Sequence Analysis Primer, M Stockton Press, New York, (1991). Methods for aligning polynucleotides or polypeptides are codified in computer programs, including the GCG program package (Devereux et al., Nuc. Acids Res. 12:387 (1984)), BLASTP, BLASTN, FASTA (Atschul et al., J. Molec. Biol. 215:403 (1990)), and Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711) which uses the local homology algorithm of Smith and Waterman (Adv. App. Math., 2:482-489 (1981)). For example, the computer program ALIGN which employs the FASTA algorithm can be used, with an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2.

[00142] When using any of the sequence alignment programs to determine whether a particular sequence is, for instance, about 95% identical to a reference sequence, the parameters are set such that the percentage of identity is calculated over the full length of the reference polynucleotide or polypeptide and that gaps in identity of up to 5% of the total number of nucleotides or amino acids in the reference polynucleotide or polypeptide are allowed.

[00143] Variants can generally be identified by modifying one of the polypeptide sequences of the invention, and evaluating the properties of the modified polypeptide to determine if it is a biological equivalent. A variant is a biological equivalent if it reacts substantially the same as a polypeptide of the invention in an assay such as an immunohistochemical assay, an enzyme-linked immunosorbent Assay (ELISA), a radioimmunoassay (RIA), immunoenzyme assay or a western blot assay, e.g. has 90-110% of the activity of the original polypeptide. In one embodiment, the assay is a competition assay wherein the biologically equivalent polypeptide is capable of reducing binding of the polypeptide of the invention to a corresponding reactive antigen or antibody by about 80, 95, 99, or 100%. An antibody that specifically binds a corresponding wild-type polypeptide also specifically binds the variant polypeptide. Variant polypeptides of the invention can comprise about 1, 2, 3, 4, 5, 10, or 20 conservative amino acid substitutions.

[00144] A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

[00145] The polypeptides described herein can further comprise a signal (or leader) sequence that co-translationally or post-translationally directs transfer of the protein. The polypeptide can also comprise a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide can be conjugated to an immunoglobulin Fc region or bovine serum albumin.

[00146] The polypeptides described herein can be covalently or non-covalently linked to an amino acid sequence to which the polypeptide is not normally associated with in nature, i.e., a heterologous amino acid sequence. A heterologous amino acid sequence can be from a non-*Anaplasma platys* organism (e.g., an Anaplasma phagocytophilum organism), a synthetic sequence, or an *Anaplasma platys* sequence not usually located at the carboxy or amino terminus of a polypeptide of the invention. Additionally, a polypeptide can be covalently or non-covalently linked to compounds or molecules other than amino acids. For example, a polypeptide can be linked to an indicator reagent, an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, a transmembrane domain, a protein purification ligand, or a combination thereof. In one embodiment of the invention a protein purification ligand can be one or more C amino acid residues at, for example, the amino terminus or carboxy terminus of a polypeptide of the invention. An amino acid spacer is a sequence of amino acids that are not usually associated with a polypeptide of the invention in nature. An amino acid spacer can comprise about 1, 5, 10, 20, 100, or 1,000 amino acids.

[00147] If desired, a polypeptide can be a fusion protein, which can also contain other amino acid sequences, such as amino acid linkers, amino acid spacers, signal sequences, TMR stop transfer sequences, transmembrane domains, as well as ligands useful in protein purification, such as glutathione-S-transferase, histidine tag, and Staphylococcal protein A, or combinations thereof. More than one polypeptide of the invention can be present in a fusion

protein. Fragments of polypeptides of the invention can be present in a fusion protein of the invention. A fusion protein of the invention can comprise one or more of *Anaplasma platys* polypeptides described herein, fragments thereof, or combinations thereof. A fusion protein can also comprise multiple copies of a same *Anaplasma platys* polypeptide or combination of different *Anaplasma platys* polypeptides described herein.

[00148] Polypeptides of the invention can be in a multimeric form. That is, a polypeptide can comprise one or more copies of an *Anaplasma platys* polypeptide of the invention or a combination thereof. A multimeric polypeptide can be a multiple antigen peptide (MAP). See e.g., Tam, J. Immunol. Methods, 196:17-32 (1996).

[00149] Polypeptides of the invention can comprise an antigen that is recognized by an antibody specific for *Anaplasma platys* P44 or *Anaplasma platys* OMP-1X. The antigen can comprise one or more epitopes (i.e., antigenic determinants). An epitope can be a linear epitope, sequential epitope or a conformational epitope. Epitopes within a polypeptide of the invention can be identified by several methods. See, e.g., U.S. Pat. No. 4,554,101; Jameson & Wolf, CABIOS 4:181-186 (1988). For example, a polypeptide of the invention can be isolated and screened. A series of short peptides, which together span an entire polypeptide sequence, can be prepared by proteolytic cleavage. By starting with, for example, 100-mer polypeptide fragments, each fragment can be tested for the presence of epitopes recognized in an ELISA. For example, in an ELISA assay an *Anaplasma platys* polypeptide, such as a 100-mer polypeptide fragment, is attached to a solid support, such as the wells of a plastic multi-well plate. A population of antibodies are labeled, added to the solid support and allowed to bind to the unlabeled antigen, under conditions where non-specific absorption is blocked, and any unbound antibody and other proteins are washed away. Antibody binding is detected by, for example, a reaction that converts a colorless substrate into a colored reaction product. Progressively smaller and overlapping fragments can then be tested from an identified 100-mer to map the epitope of interest.

[00150] The polypeptides described herein can be produced recombinantly. A polynucleotide encoding a polypeptide described herein can be introduced into a recombinant expression vector, which can be expressed in a suitable expression host cell system using techniques well known in the art. A variety of bacterial, yeast, plant, mammalian, and insect expression systems are available in the art and any such expression system can be used.

Optionally, a polynucleotide encoding a polypeptide can be translated in a cell-free translation system. A polypeptide can also be chemically synthesized or obtained from *Anaplasma platys* cells.

[00151] For example, and not to be limiting, an immunogenic polypeptide of the invention can comprise an amino acid sequence shown in SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or fragments thereof. An immunogenic polypeptide can elicit antibodies or other immune responses (e.g., T-cell responses of the immune system) that recognize epitopes of a polypeptide having SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or fragments thereof. An immunogenic polypeptide of the invention can also be a fragment of a polypeptide that has an amino acid sequence shown in SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8. An immunogenic polypeptide

fragment of the invention can be about 10, 15, 20, 25, 30, 40, 50 or more amino acids in length.

***Polypeptide Production***

[00152] Polypeptides that can be used in the disclosed methods can be produced by any method known in the art. One method of producing the disclosed polypeptides is to link two or more amino acid residues, peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides are chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (tert -butyloxycarbonoyl) chemistry (Applied Biosystems, Inc., Foster City, CA). A peptide or polypeptide can be synthesized and not cleaved from its synthesis resin, whereas the other fragment of a peptide or protein can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group, which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, (Grant GA (1992) *Synthetic Peptides: A User Guide.* W.H. Freeman and Co., N.Y. (1992); Bodansky M and Trost B., Ed. (1993) *Principles of Peptide Synthesis.* Springer-Verlag Inc., NY). Alternatively, the peptide or polypeptide is independently synthesized *in vivo*. Once isolated, these independent peptides or polypeptides can be linked to form a peptide or fragment thereof via similar peptide condensation reactions.

[00153] For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen L et al., *Biochemistry*, 30:4151 (1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. This method consists of a two-step chemical reaction (Dawson et al. *Science*, 266:776-779 (1994)). The first step is the chemoselective reaction of an unprotected synthetic peptide-thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester- linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site (Baggiolim M et al. (1992) *FEBS Lett.* 307:97-101; Clark-Lewis I *et al.*, *J.Biol.Chem.*, 269:16075 (1994); Clark-Lewis I et al., *Biochem.*, 30:3128 (1991); Rajarathnam K et al., *Biochem.* 33:6623-30 (1994)).

[00154] Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer, M et al. *Science*, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC et al., *Techniques in Protein Chemistry IV*. Academic Press, New York, pp. 257-267 (1992)).

[00155] Also disclosed are the components to be used to prepare the disclosed *A. platys* peptides that can be used in the disclosed methods as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular polynucleotide is disclosed and discussed and a number of modifications that can be made to a number of molecules including the polynucleotide are discussed, specifically contemplated is each and every combination and permutation of polynucleotide and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

[00156] It is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed genes and proteins herein is through defining the variants and derivatives in terms of homology to specific known sequences. Specifically disclosed are variants of the genes and proteins herein disclosed which have at least, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95,

96, 97, 98, or 99 percent homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins or nucleic acids, such as genes. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

***Antibodies***

[00157] Described herein are isolated or purified antibodies that selectively hybridize to a peptide chosen from: *Anaplasma platys* P44 protein, *Anaplasma platys* OMP-1X protein, P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof. In one aspect, the antibodies described herein can hybridize to a peptide chosen from one or more of: P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure

19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, OMP-1X Box 1, OMP-1X Box 2, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof. In a further aspect, the antibodies described herein can hybridize to one or more of a peptide that is at least 95% identical to the P44 or OMP-1X peptide sequences described herein. In a further aspect, the antibodies described herein can hybridize to one or more of a variant or antigenic fragment of the P44 or OMP-1X peptide sequences described herein. In still a further aspect, the antibodies described herein can hybridize to one or more of a variant or antigenic fragment of a peptide that is at least 95% identical to the P44 or OMP-1X peptide sequences described herein.

[00158] Disclosed herein are antibodies that specifically and stably bind to an *Anaplasma platys* p44 polypeptide, an *Anaplasma platys* OMP-1X peptide, or fragment thereof. Antibodies can also specifically and stably bind to an *Anaplasma platys* P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6 polypeptide or fragment thereof. Antibodies can also specifically and stably bind to an *Anaplasma platys* OMP-1X Box 1 polypeptide, an OMP-1X Box 2 polypeptide or fragment thereof. One of skill in the art can easily determine if an antibody is specific for an *Anaplasma platys* polypeptide using assays described herein. An antibody can be a polyclonal antibody, a monoclonal antibody, a single chain antibody (scFv), or an antigen binding fragment of an antibody. Antigen-binding fragments of antibodies are a portion of an intact antibody comprising the antigen binding site or variable region of an intact antibody, wherein the portion is free of the constant heavy chain domains of the Fc region of the intact antibody. Examples of antibody fragments include Fab, Fab', Fab'-SH, $F(ab')_2$ and $F_v$ fragments.

[00159] The antibodies described herein can be any antibody class, including for example, IgG, IgM, IgA, IgD and IgE. An antibody or fragment thereof binds to an epitope of a polypeptide of the invention. An antibody can be made in vivo in suitable laboratory animals or in vitro using recombinant DNA techniques. Means for preparing and characterizing antibodies are well know in the art. See, e.g., Dean, Methods Mol. Biol. 80:23-37 (1998); Dean, Methods Mol. Biol. 32:361-79 (1994); Baileg, Methods Mol. Biol. 32:381-88 (1994); Gullick, Methods Mol. Biol. 32:389-99 (1994); Drenckhahn et al. Methods Cell. Biol. 37:7-56 (1993); Morrison, Ann. Rev. Immunol. 10:239-65 (1992); Wright et al. Crit. Rev. Immunol. 12:125-68 (1992). For example, polyclonal antibodies can be produced by administering a polypeptide of the invention to an animal, such as a human or other primate, mouse, rat, rabbit, guinea pig, goat, pig, dog, cow, sheep, donkey, or horse. Serum from the immunized animal is collected and the antibodies are purified from the plasma by, for example, precipitation with ammonium sulfate, followed by chromatography, such as affinity chromatography. Techniques for producing and processing polyclonal antibodies are known in the art.

[00160] "Specifically binds" or "specific for" means that a first antigen, e.g., an *Anaplasma platys* polypeptide, recognizes and binds to an antibody of the invention with greater affinity than other, non-specific molecules. A non-specific molecule is an antigen that shares no common epitope with the first antigen. In this case, *Anaplasma platys* polypeptides would not generally be desirable choices for non-specific control molecules. For example, an antibody raised against a first antigen (e.g., a polypeptide) to which it binds more efficiently than to a non-specific antigen can be described as specifically binding to the first antigen. In a preferred embodiment, an antibody or antigen-binding portion thereof specifically binds to a polypeptide of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or fragments thereof. Specific binding can be tested using, for example, an enzyme-linked immunosorbant assay (ELISA),

a radioimmunoassay (RIA), or a western blot assay using methodology well known in the art.

[00161] Additionally, monoclonal antibodies directed against epitopes present on a polypeptide of the invention can also be readily produced. For example, normal B cells from a mammal, such as a mouse, which was immunized with a polypeptide of the invention can be fused with, for example, HAT-sensitive mouse myeloma cells to produce hybridomas. Hybridomas producing *Anaplasma platys* -specific antibodies can be identified using RIA or ELISA and isolated by cloning in semi-solid agar or by limiting dilution. Clones producing *Anaplasma platys* -specific antibodies are isolated by another round of screening. Monoclonal antibodies can be screened for specificity using standard techniques, for example, by binding a polypeptide of the invention to a microtiter plate and measuring binding of the monoclonal antibody by an ELISA assay. Techniques for producing and processing monoclonal antibodies are known in the art. See e.g., Kohler & Milstein, Nature, 256:495 (1975). Particular isotypes of a monoclonal antibody can be prepared directly, by selecting from the initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of a different isotype by using a sib selection technique to isolate class-switch variants. See Steplewski et al., P.N.A.S. U.S.A. 82:8653 1985; Spria et al., J. Immunolog. Meth. 74:307, 1984. Monoclonal antibodies of the invention can also be recombinant monoclonal antibodies. See, e.g., U.S. Pat. No. 4,474,893; U.S. Pat. No. 4,816,567. Antibodies of the invention can also be chemically constructed. See, e.g., U.S. Pat. No. 4,676,980.

[00162] Antibodies can be chimeric (see, e.g., U.S. Pat. No. 5,482,856), humanized (see, e.g., Jones et al., Nature 321:522 (1986); Reichmann et al., Nature 332:323 (1988); Presta, Curr. Op. Struct. Biol. 2:593 (1992)), caninized, canine, or human antibodies. Human antibodies can be made by, for example, direct immortilization, phage display, transgenic mice, or a Trimera methodology, see e.g., Reisener et al., Trends Biotechnol. 16:242-246 (1998).

[00163] Antibodies that specifically bind *Anaplasma platys* antigens (e.g., *Anaplasma platys* polypeptides), are particularly useful for detecting the presence of Apl or Apl antigens in a sample, such as a serum, blood, plasma, urine, fecal, or saliva sample from an Apl- or Aph-infected animal. An immunoassay for *Anaplasma platys* antigen can utilize one

antibody or several antibodies. An immunoassay for *Anaplasma platys* antigen can use, for example, a monoclonal antibody specific for an *Anaplasma platys* epitope, a combination of monoclonal antibodies specific for epitopes of one *Anaplasma platys* polypeptide, monoclonal antibodies specific for epitopes of different *Anaplasma platys* polypeptides, polyclonal antibodies specific for the same *Anaplasma platys* antigen, polyclonal antibodies specific for different *Anaplasma platys* antigens, or a combination of monoclonal and polyclonal antibodies. Immunoassay protocols can be based upon, for example, competition, direct reaction, or sandwich type assays using, for example, labeled antibody. Antibodies can be labeled with any type of label known in the art, including, for example, fluorescent, chemiluminescent, radioactive, enzyme, colloidal metal, radioisotope and bioluminescent labels.

[00164] Antibodies or fragments thereof can be bound to a support and used to detect the presence of *Anaplasma platys* antigen. Supports include, for example, glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magletite.

[00165] Antibodies can further be used to isolate *Anaplasma platys* organisms or *Anaplasma platys* antigens by immunoaffinity columns. The antibodies can be affixed to a solid support by, for example, adsorbtion or by covalent linkage so that the antibodies retain their immunoselective activity. Optionally, spacer groups can be included so that the antigen binding site of the antibody remains accessible. The immobilized antibodies can then be used to bind *Anaplasma platys* organisms or *Anaplasma platys* antigens from a sample, such as a biological sample including saliva, serum, sputum, blood, urine, feces, cerebrospinal fluid, amniotic fluid, wound exudate, or tissue. The bound *Anaplasma platys* organisms or *Anaplasma platys* antigens are recovered from the column matrix by, for example, a change in pH.

[00166] Antibodies can also be used in immunolocalization studies to analyze the presence and distribution of a polypeptide of the invention during various cellular events or physiological conditions. Antibodies can also be used to identify molecules involved in passive immunization and to identify molecules involved in the biosynthesis of non-protein antigens. Identification of such molecules can be useful in vaccine development. Antibodies, including, for example, monoclonal antibodies and single chain antibodies, can

be used to monitor the course of amelioration of a disease caused by *Anaplasma platys*. By measuring the increase or decrease of *Anaplasma platys* antibodies to *Anaplasma platys* antigens in a test sample from an animal, it can be determined whether a particular therapeutic regiment aimed at ameliorating the disorder is effective. Antibodies can be detected and/or quantified using for example, direct binding assays such as RIA, ELISA, or western blot assays.

[00167] In one aspect, the antibodies can be immunoglobulin molecules that specifically and stably bind to *A. platys* P44 or OMP-1X polypeptide or fragment thereof. In a further aspect, the antibody can be monoclonal, polyclonal, or a single chain antibody. In yet a further aspect, an antibody can be an antigen-binding fragments, which is a portion of an intact antibody comprising the antigen binding site or variable region of an intact antibody, wherein the portion is free of the constant heavy chain domains of the Fc region of the intact antibody.

[00168] In one aspect, monoclonal antibodies directed against epitopes present on a polypeptide discussed herein can be produced. In a further aspect, clones producing *A. platys*-specific antibodies can be isolated via additional screening. In yet a further aspect, monoclonal antibodies can also be recombinant monoclonal antibodies. Monoclonal antibodies can be screened for specificity using standard techniques known in the art.

[00169] In one aspect, an antibody can belong to any antibody class. In a further aspect, an antibody or fragment thereof can bind to an epitope of a polypeptide disclosed herein. An antibody can be made *in vivo* in suitable laboratory animals or *in vitro* via recombinant DNA techniques known in the art.

[00170] Means for preparing and characterizing antibodies are well known in the art. For example, polyclonal antibodies can be produced by administering a polypeptide described herein to an animal, such as a human or other primate, mouse, rat, rabbit, dog, cow, sheep, or horse. Serum from the immunized animal can be collected and the antibodies can be purified from the plasma.

[00171] In one aspect, antibodies can be chimeric, canine, or human antibodies. In a further aspect, antibodies or fragments thereof can be bound to a support. Supports can include, glass, polystyrene, polypropylene, polyethylene, nylon, celluloses, or polyacrylamides.

*Vaccines*

[00172] In one aspect, described herein are *Anaplasma platys* P44 protein based vaccines. Thus, described herein are peptides comprising one or more amino-acid sequences selected from the group consisting of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof. In a further aspect, the peptides disclosed herein can comprise one or more amino-acid sequences selected from the group consisting of a combination of any P44 peptide sequences described herein.

[00173] In yet a further aspect, the vaccines described herein can comprise one or more amino-acid sequences selected from the group consisting of a variant of the P44 proteins described herein, or an antigenic fragment of the P44 proteins described herein. In still a further aspect, the vaccines described herein can comprise one or more amino-acid sequences selected from the group consisting of a sequence that is at least 95% identical to a P44 protein, a variant of the P44 protein, or an antigenic fragment of the P44 protein sequences described herein. The peptides described herein can also be any antigenically related variant of the peptide sequences which have an identity of 95% and are capable of immunologically mimicking the corresponding antigenic determinant site of the P44 protein of *Anaplasma platys*.

[00174] In one aspect, the vaccines described herein can be *Anaplasma platys* OMP-1X protein based vaccines. Thus, described herein are peptides comprising one or more amino-acid sequences selected from the group consisting of OMP-1X Box 1, OMP-1X Box 2, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof. In a further aspect, the peptides disclosed herein can comprise one or more amino-acid sequences selected from the group consisting of a combination of any OMP-1X peptide sequences described herein.

[00175] In yet a further aspect, the vaccines described herein can comprise one or more amino-acid sequences selected from the group consisting of a variant of the OMP-1X proteins described herein, or an antigenic fragment of the OMP-1X proteins described herein. In still a further aspect, the vaccines described herein can comprise one or more amino-acid sequences selected from the group consisting of a sequence that is at least 95% identical to a OMP-1X protein, a variant of the OMP-1X protein, or an antigenic fragment of the OMP-1X protein sequences described herein. The peptides described herein can also be any

antigenically related variant of the peptide sequences which have an identity of 95% and are capable of immunologically mimicking the corresponding antigenic determinant site of the OMP-1X protein of *Anaplasma platys*. Antigenically related variants can have amino acids added, inserted, substituted or deleted.

[00176] Furthermore, described herein are chimeric peptides comprising: one or more *Anaplasma platys* P44 proteins or *Anaplasma platys* OMP-1X proteins described herein; one or more variants of the *Anaplasma platys* P44 proteins or *Anaplasma platys* OMP-1X proteins described herein; one or more antigenic fragments of the *Anaplasma platys* P44 proteins or *Anaplasma platys* OMP-1X proteins described herein; or one or more proteins that are at least 95% identical to the *Anaplasma platys* P44 proteins or *Anaplasma platys* OMP-1X proteins described herein, linked to a carrier polypeptide that can comprise at least one T-cell epitope. In one aspect, the chimeric peptides described herein can further comprise a purification tag peptide sequence. For example, and not to be limiting, the purification tag sequence can be a Histidine-tag sequence. Also disclosed herein are purified antibodies that are immunospecific to the chimeric peptides described herein. In one aspect, a purification tag peptide sequence (such as a Histidine tag or a Glutathione-S-transferase tag) can be used in order to aid subsequent purification of the polypeptide. Optional short peptide spacer sequences can be introduced between elements of the chimeric polypeptide. When one is required a Histidine tag sequence can be located at the C-terminus of the polypeptide.

[00177] Further described herein are vaccine compositions comprising an immunogenic amount of at least: one *Anaplasma platys* P44 protein or *Anaplasma platys* OMP-1X protein described herein; one variant of the *Anaplasma platys* P44 proteins or *Anaplasma platys* OMP-1X proteins described herein; one antigenic fragment of the *Anaplasma platys* P44 proteins or *Anaplasma platys* OMP-1X proteins described herein; or one protein that is at least 95% identical to the *Anaplasma platys* P44 proteins or *Anaplasma platys* OMP-1X proteins described herein, wherein the protein or peptide can be in a pharmaceutically acceptable excipient, and an optional adjuvant. Vaccine preparation is generally described in Vaccine Design ("The subunit and adjuvant approach" (eds. Powell M. F. & Newman M. J). (1995) Plenum Press New York), which is hereby incorporated in its entirety by this reference. Suitable adjuvants include, but are not limited to an aluminium salt such as aluminium hydroxide gel (alum) or aluminium phosphate, but can also be a salt of calcium,

iron or zinc, or can be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatised polysaccharides, or polyphosphazenes. Other known adjuvants include CpG containing oligonucleotides. The oligonucleotides can be characterized in that the CpG dinucleotide is unmethylated. Such oligonucleotides are well known in the art and are described in, for example WO96/02555. In one aspect, the adjuvants can induce an immune response, for example, of the TH1 type. High levels of Th1-type cytokines can favor the induction of cell mediated immune responses to the given antigen, while high levels of Th2-type cytokines can favor the induction of humoral immune responses to the antigen. Suitable adjuvant systems can include, for example, monophosphoryl lipid A, 3-de-O-acylated monophosphoryl lipid A (3D-MPL), or a combination of 3D-MPL together with an aluminium salt. CpG oligonucleotides can also induce a TH1 response. An enhanced system can involve the combination of a monophosphoryl lipid A and a saponin derivative, for example, the combination of QS21 and 3D-MPL as disclosed in WO 94/00153, or a less reactogenic composition where the QS21 can be quenched with cholesterol as described in WO 96/33739. Another adjuvant formulation involving QS21 3D-MPL & tocopherol in an oil in water emulsion is described in WO 95/17210.

[00178] Also described herein are methods of inducing an immune response in a mammal susceptible to *Anaplasma platys* infection comprising administering to the mammal an effective amount of the vaccine compositions described herein. As used herein, "infection" can also mean "exposure," and the terms can be used interchangeably.

[00179] Additionally, described herein are methods of preventing *Anaplasma platys* infection comprising administering to a mammal an effective amount of the vaccine compositions described herein.

***Vectors***

[00180] Also described herein are vectors for transformation of a host cell comprising an isolated polynucleotide that can encode an outer membrane protein of *Anaplasma platys*, a variant of said outer membrane protein, or an immunogenic fragment of said outer membrane protein. In one aspect, the outer membrane protein can be the P44 protein, P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, OMP-1X protein, OMP-1X Box 1, OMP-1X Box 2, OMP-1X Box 1 and OMP-1X Box 2, or a fragment thereof. The vectors

disclosed herein can comprise any of the isolated polynucleotide sequences disclosed or described herein.

[00181] The polynucleotides described herein can comprise coding sequences for naturally occurring polypeptides or can encode altered sequences that do not occur in nature. If desired, polynucleotides can be cloned into an expression vectors comprising expression control elements, including for example, origins of replication, promoters, enhancers, or other regulatory elements that drive expression of the polynucleotides of the invention in host cells. An expression vector can be, for example, a plasmid, such as pBR322, pUC, or ColE1, or an adenovirus vector, such as an adenovirus Type 2 vector or Type 5 vector. Optionally, other vectors can be used, including but not limited to Sindbis virus, simian virus 40, alphavirus vectors, poxvirus vectors, and cytomegalovirus and retroviral vectors, such as murine sarcoma virus, mouse mammary tumor virus, Moloney murine leukemia virus, and Rous sarcoma virus. Minichromosomes such as MC and MC1, bacteriophages, phagemids, yeast artificial chromosomes, bacterial artificial chromosomes, virus particles, virus-like particles, cosmids (plasmids into which phage lambda cos sites have been inserted) and replicons (genetic elements that are capable of replication under their own control in a cell) can also be used.

[00182] Methods for preparing polynucleotides operably linked to an expression control sequence and expressing them in a host cell are well-known in the art. See, e.g., U.S. Pat. No. 4,366,246. A polynucleotide of the invention is operably linked when it is positioned adjacent to or close to one or more expression control elements, which direct transcription and/or translation of the polynucleotide.

[00183] In a further aspect, the vectors described herein can be used in a process for making a corresponding outer membrane protein of *Anaplasma platys*, a variant of said outer membrane protein, or an immunogenic fragment of said outer membrane protein. For example, and not to be limiting, the process can comprise transfecting host cells with any of the vectors described herein and inducing expression of the outer membrane protein or the variant or immunogenic fragment thereof in any of the host cells described herein.

[00184] Expression vectors for production of proteins and peptides are well known in the art (see Ausubel et al., 2004, Current Protocols In Molecular Biology, Greene Publishing and Wiley-Interscience, New York). Such expression vectors can include the nucleic acid

sequence encoding the *Anaplasma platys* polypeptides linked to regulatory elements, such as a promoter, which drives transcription of the DNA and can be adapted for expression in prokaryotic (e.g., E. coli) and eukaryotic (e.g., yeast, insect or mammalian cells) hosts. A variant *Anaplasma platys* polypeptide can also be expressed in an expression vector in which a variant *Anaplasma platys* gene is operably linked to a promoter. The promoter can be a eukaryotic promoter for expression in a mammalian cell. The transcription regulatory sequences can comprise a heterologous promoter and optionally an enhancer, which is recognized by the host cell. Commercially available expression vectors can also be used. Expression vectors can include host-recognized replication systems, amplifiable genes, selectable markers, host sequences useful for insertion into the host genome, and the like.

***Host Cells***

[00185] Also disclosed herein are host cells comprising any of the vectors disclosed or described herein. Suitable host cells can include, but are not limited to, bacteria such as E. coli, yeast, filamentous fungi, mollusk cells, snail cells, insect cells, and mammalian cells, which are typically immortalized, including mouse, hamster, human, and monkey cell lines, and derivatives thereof. Host cells may be able to process the *Anaplasma platys* gene product to produce an appropriately processed, mature polypeptide. Such processing can include glycosylation, ubiquitination, disulfide bond formation, and the like.

***Kits***

[00186] Described herein are kits for diagnosing *Anaplasma platys* in a subject, wherein the kit can comprise the *Anaplasma platys* P44 protein, an antigenic fragment of the *Anaplasma platys* P44 protein, or both. In one aspect, in the kits disclosed herein, the protein can comprise: one or more *Anaplasma platys* P44 proteins described herein; one or more variants of the *Anaplasma platys* P44 proteins described herein; one or more antigenic fragments of the *Anaplasma platys* P44 proteins described herein; or one or more proteins that are at least 95% identical to the *Anaplasma platys* P44 proteins described herein.

[00187] Also described herein are kits for diagnosing *Anaplasma platys* in a subject, wherein the kit can comprise the *Anaplasma platys* OMP-1X protein, an antigenic fragment of the *Anaplasma platys* OMP-1X protein, or both. In one aspect, in the kits disclosed herein, the protein can comprise: one or more *Anaplasma platys* OMP-1X proteins described herein; one or more variants of the *Anaplasma platys* OMP-1X proteins described herein; one

or more antigenic fragments of the *Anaplasma platys* OMP-1X proteins described herein; or one or more proteins that are at least 95% identical to the *Anaplasma platys* OMP-1X proteins described herein.

[00188] Further described herein are kits for diagnosing *Anaplasma platys* in a subject comprising one or more of the antibodies described herein. In one aspect, the kits described herein can further comprise a biomolecule for detecting an interaction between the reagent and antibodies in a sample from an animal.

[00189] Also described herein are reagent kits for diagnosing infection or exposure with *Anaplasma platys* in a subject comprising a DNA probe or primer constructed to correspond to the P44 protein, P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, or P44 Box 6 of *Anaplasma platys*, characterized in that the probe or primer comprises one or more of the nucleotides or polynucleotides described herein.

[00190] Also described herein are reagent kits for diagnosing infection or exposure with *Anaplasma platys* in a subject comprising a DNA probe or primer constructed to correspond to the OMP-1X protein, OMP-1X Box 1, or OMP-1X Box 2 of *Anaplasma platys*, characterized in that the probe or primer comprises one or more of the nucleotides or polynucleotides described herein.

[00191] The kits described herein can comprise one or more of the polypeptides described herein and means for determining binding of the polypeptide to anti- *Anaplasma platys* or antibody fragments in the sample. A kit or article of manufacture can also comprise one or more antibodies or antibody fragments described herein and means for determining binding of the antibodies or antibody fragments to *Anaplasma platys* and/or *Anaplasma platys* polypeptides in the sample. A kit can comprise a device containing one or more polypeptides or antibodies described herein and instructions for use of the one or more polypeptides or antibodies for, e.g., the identification of an *Anaplasma platys* infection in a mammal. The kit can also comprise packaging material comprising a label that indicates that the one or more polypeptides or antibodies of the kit can be used for the identification of *Anaplasma platys* infection. Other components such as buffers, controls, and the like, known to those of ordinary skill in art, can be included in such test kits. The polypeptides, antibodies, assays, and kits of the invention are useful, for example, in the diagnosis of individual cases of *Anaplasma platys* infection in a subject, as well as epidemiological studies of *Anaplasma*

*platys* outbreaks. Exposure to *Anaplasma platys* can also be detected. Exposure would include the presence of *Anaplasma platys* organisms without clinical symptoms and prior infection with *Anaplasma platys*.

***Samples***

[00192] Vertebrate host samples are collected from body tissue or bodily fluid, such as for example, blood, plasma, saliva, and peripheral blood mononuclear cells. For the invertebrate vectors which can transmit the pathogen from one vertebrate host to another, the sample can be from dissected ticks (*e.g.*, midgut, salivary glands, and hemolymph), tick pieces, and frozen and smashed ticks in preparation for PCR assays. Further preparation of tick tissues can involve heating the sample, digesting the samples with proteases, and isolating pure DNA from the tick tissues. Other suitable samples include, but are not limited to, saliva, cheek scrapings, biopsies of retina, kidney or liver or other organs or tissues; skin biopsies; amniotic fluid; or CNS samples; and the like.

## METHODS

***PCR Based Diagnostics***

[00193] Described herein are methods for detecting *Anaplasma platys* in a sample obtained from a subject, comprising (a) providing a primer set comprising: (i) one or more forward primers comprising the sequence of: SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO:84, or SEQ ID NO: 90 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO:88, SEQ ID NO: 89 or SEQ ID NO: 91; (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and (c) determining the length or sequence of the PCR products of step (b), wherein the presence of a PCR product having a length or sequence which corresponds to the length or sequence, respectively, of that region of the *Anaplasma platys* p44 gene which is located between the regions to which the one or more forward primers and the one or more reverse primers bind is indicative of the presence of *Anaplasma platys* in the sample. For example, and not to be limiting, the one or more forward or reverse primers can be from 15 to 35 nucleotides in length. In one aspect, the forward and the reverse primer can comprise SEQ ID NO: 90 and SEQ ID NO: 91, respectively.

[00194] Further described herein are primer sets for detecting *Anaplasma platys* in a sample, the primer set comprising: (a) one or more forward primers comprising the sequence of: SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO:84, or SEQ ID NO: 90 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO:88, SEQ ID NO: 89 or SEQ ID NO: 91. For example, and not to be limiting, the one or more forward or reverse primers can be from 15 to 35 nucleotides in length. In one aspect, the forward and the reverse primer can comprise SEQ ID NO: 90 and SEQ ID NO: 91, respectively.

[00195] Also described herein are methods for detecting *Anaplasma platys* in a sample obtained from a subject, comprising (a) providing a primer set comprising: (i) one or more forward primers comprising the sequence of: SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO:56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO:57, SEQ ID NO: 59, SEQ ID NO: 61, or SEQ ID NO: 63; (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and (c) determining the length or sequence of the PCR products of step (b), wherein the presence of a PCR product having a length or sequence which corresponds to the length or sequence, respectively, of that region of the *Anaplasma platys* p44 gene which is located between the regions to which the one or more forward primers and the one or more reverse primers bind is indicative of the presence of *Anaplasma platys* in the sample. For example, and not to be limiting, the one or more forward or reverse primers can be from 15 to 35 nucleotides in length. In one aspect, the forward and the reverse primer can comprise one or more of pairs of sequences described herein, including, but not limited to: PAIR 1: SEQ ID NO: 52 and SEQ ID NO: 53; PAIR 2: SEQ ID NO: 54 and SEQ ID NO: 55; PAIR 3: SEQ ID NO: 56 and SEQ ID NO: 57; PAIR 4: SEQ ID NO: 58 and SEQ ID NO: 59; PAIR 5: SEQ ID NO: 60 and SEQ ID NO: 61; or PAIR 6: SEQ ID NO: 62 and SEQ ID NO: 63.

[00196] Further described herein are primer sets for detecting *Anaplasma platys* in a sample, the primer set comprising: (a) one or more forward primers comprising the sequence of: SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO:84, or SEQ ID NO: 90 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO:88, SEQ ID NO: 89 or SEQ ID NO: 91. For example, and not to be limiting, the one or more forward or reverse primers can be from 15 to 35 nucleotides in length.

[00197] Described herein are methods for detecting *Anaplasma platys* in a sample obtained from a subject, comprising (a) providing a primer set comprising: (i) one or more forward primers comprising the sequence of: SEQ ID NO: 64, SEQ ID NO: 18,or SEQ ID NO: 20 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 65, SEQ ID NO: 19 , or SEQ ID NO: 99; (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and (c) determining the length or sequence of the PCR products of step (b), wherein the presence of a PCR product having a length or sequence which corresponds to the length or sequence, respectively, of that region of the *Anaplasma platys* OMP-1X gene which is located between the regions to which the one or more forward primers and the one or more reverse primers bind is indicative of the presence of *Anaplasma platys* in the sample. For example, and not to be limiting, the one or more forward or reverse primers can be from 15 to 35 nucleotides in length. In one aspect, the forward and reverse primers can comprise one or more pairs of sequences described herein, including, but not limited to: PAIR 1: SEQ ID NO: 64 and SEQ ID NO: 65; PAIR 2: SEQ ID NO: 18 and SEQ ID NO: 19; or PAIR 3: SEQ ID NO: 20 and SEQ ID NO: 99, respectively.

[00198] Further described herein are primer sets for detecting *Anaplasma platys* in a sample, the primer set comprising: (a) one or more forward primers comprising the sequence of: SEQ ID NO: 64, SEQ ID NO: 18, or SEQ ID NO: 20 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 65, SEQ ID NO: 19, or SEQ ID NO: 99. For example, and not to be limiting, the one or more forward or reverse primers can be from 15 to 35 nucleotides in length.

[00199] Also described herein are methods of detecting the presence of *Anaplasma platys* in a sample by contacting the sample with a DNA probe or primer constructed to correspond to the P44 protein of *Anaplasma platys*, characterized in that the probe or primer comprises one or more of the nucleotides or polynucleotides described herein.

[00200] Further described herein are methods of detecting the presence of *Anaplasma platys* in a sample by contacting the sample with a DNA probe or primer constructed to correspond to the OMP-1X protein, OMP-1X Box 1, or OMP-1X Box 2 of *Anaplasma platys*, characterized in that the probe or primer comprises one or more of the nucleotides or polynucleotides described herein.

[00201] PCR assays are well known in the art, including, for example, U.S. Pat. Nos. 4,683,195; U.S. Pat. No. 4,683,202;U.S. Pat. No. 4,965,188. Generally, polynucleotide primers are annealed to denatured strands of a target nucleic acid. Primer extension products are formed by polymerization of deoxynucleoside triphosphates by a polymerase. PCR then involves repetitive cycles of template nucleic acid denaturation, primer annealing and extension of the annealed primers by the action of a thermostable polymerase. The process results in exponential amplification of the target *Anaplasma platys* nucleic acids in the test sample, which allows for the detection of target polynucleotides existing in very low concentrations in a sample.

[00202] Real-time PCR assays are based on the detection of a signal, e.g., a fluorescent reporter signal. This signal increases in direct proportion to the amount of PCR product in a reaction. Real-time PCR is any amplification technique that makes it possible to monitor the evolution of an ongoing amplification reaction. See, Quantitation of DNA/RNA Using Real-Time PCR Detection, Perkin Elmer Applied Biosystems (1999); PCR Protocols (Academic Press New York, 1989). By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during exponential phase where the first significant increase in the amount of PCR product correlates to the initial amount of target template. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed.

[00203] Described herein are methods for detecting and/or quantifying *Anaplasma platys* polynucleotides in a test sample. Sense primers and antisense primers can be added to a test sample under conditions suitable for a polymerase chain reaction. The primers hybridize with *Anaplasma platys P44 or OMP-1X* polynucleotides such that an amplification product is formed if *Anaplasma platys P44 or OMP-1X* polynucleotides are present in the test sample. In one aspect, the primers can be SEQ ID NOs: 90 and 91. Amplification products are detected and the presence and/or quantity of *Anaplasma platys P44 or OMP-1X* polynucleotides is determined. Amplification products can be detected with a polynucleotide probe that hybridizes, under conditions suitable for a polymerase chain reaction, with an *Anaplasma platys P44 or OMP-1X* polynucleotide sequence. Examples of probes include SEQ ID NOs: 17 which can be used to identify the presence of an OMP-1X polynucleotide . The amplification product can be quantified by measuring a detection signal from the probe and comparing said detection signal to a second probe detection

signal from a quantification standard. The quantification standard can be extracted in parallel with the test sample.

[00204] Also disclosed are methods wherein the PCR primers can be selected from the variable regions of an *Anaplasma platys P44 or OMP-1X* polynucleotide. For example, primers of 10, 15, 20, 25, 30, or 40 contiguous nucleotides can be selected from the regions of P44 Boxes 1-6 or OMP-1X Boxes 1 or 2.

[00205] The polynucleotides described herein can be used to detect the presence of *Anaplasma platys* polynucleotides in a sample. The polynucleotides can be used to detect *Anaplasma platys* polynucleotides in a sample by a simple hybridization reaction and can also be used in, e.g., polymerase chain reactions (PCR) such as a real-time PCR reaction. The methods and compositions described herein can also be used to differentially detect the presence *Anaplasma platys* from *Anaplasma phagocytophilum or other Anaplasma* species.

***Antibody Based Diagnostics***

[00206] Also described herein are methods for detecting *Anaplasma platys* in a sample by contacting the sample with one or more of the antibodies described herein. In one aspect, to the *Anaplasma platys* in a sample of a bodily fluid from a patient. The method comprises providing an isolated outer membrane protein of *Anaplasma platys*, for example, a recombinant form of the isolated protein, contacting the outer membrane protein or polypeptide with a sample taken from the patient; and assaying for the formation of a complex between the outer membrane protein or polypeptide and antibodies in the sample. In one aspect, the isolated protein or polypeptide be attached to a substrate such as a column, plastic dish, matrix, or membrane, preferably nitrocellulose. The sample can be a tissue or a biological fluid, including urine, whole blood, exudate, or serum. The sample can be untreated, subjected to precipitation, fractionation, separation, or purification before combining with the isolated protein or peptide. Interactions between antibodies in the sample and the isolated protein or peptide can be detected by radiometric, colorimetric, or fluorometric means, size-separation, or precipitation. In one aspect, detection of the antibody-outer membrane protein complex can be by addition of a secondary antibody that can be coupled to a detectable tag, such as for example, an enzyme, fluorophore, or chromophore. Formation of the complex is indicative of the presence of anti- *Anaplasma platys* antibodies,

either IgM or IgG, in the patient. Thus, the method can be used to determine whether a subject is infected with *Anaplasma platys*.

[00207] In one aspect, the method can employ an enzyme-linked immunosorbent assay (ELISA) or a Western immunoblot procedure. Such methods can be relatively simple to perform and do not require special equipment as long as membrane strips are coated with a high quality antigen. Accordingly, in one aspect, it can be advantageous to use a recombinant form of the outer membrane protein of *Anaplasma platys* since such proteins, typically, are more pure and consistent in quality than a purified form of such protein.

***Peptide Based Diagnostics***

[00208] Described herein are methods of detecting antibodies that specifically bind an *Anaplasma platys* polypeptide, comprising: (a) contacting a purified polypeptide comprising the amino acid sequence of one or more of the following: (i) P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof; (ii) a variant of P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof; (iii) an antigenic fragment of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof; (iv) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof; (v) a variant of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof; (vi) an antigenic fragment of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45; (vii) SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof; (viii) a variant of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof; (ix) an antigenic fragment of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ

ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, or SEQ ID NO: 97; (x) the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof; (xi) a variant of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure

23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof; (xii) an antigenic fragment of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof; (xii) a combination of one or more of the sequences in (i) – (xii); (xiv) OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof; (xv) a variant of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof; (xvi) an antigenic fragment of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof; (xvii) SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof; (xviii) a variant of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof; (xix) an antigenic fragment of

SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or SEQ ID NO: 9; (xx) SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof; (xxi) a variant of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof; (xxii) an antigenic fragment of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, or SEQ ID NO: 8; (xxiii) the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; (xxiv) a variant of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; (xxv) an antigenic fragment of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; or (xxvi) a combination of one or more of the sequences in (xiv) – (xxv); with a test sample, under conditions that allow polypeptide/antibody complex to form; (b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that antibodies specific for an *Anaplasma platys* polypeptide is present in the test sample. For example, and not to be limiting, the test sample can be a biological sample from a subject, and the detection

of polypeptide/antibody complexes can be an indication that the subject has an *Anaplasma platys* infection or has been exposed to *Anaplasma platys*.

[00209] In one aspect, the methods of detecting antibodies that specifically bind an *Anaplasma platys* polypeptide described herein can further comprise determining the amount of antibody in the test sample. In yet a further aspect, the purified polypeptide can be attached to a substrate. In still a further aspect, the purified protein can be a fusion protein. For example, and not to be limiting, the purified polypeptide can be fused to an indicator reagent, an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, a transmembrane domain, a protein purification ligand, a heterologous protein, or a combination thereof. In a further aspect, the purified polypeptide can be in multimeric form.

[00210] In yet a further aspect, the methods of detecting antibodies that specifically bind an *Anaplasma platys* polypeptide described herein can further comprise a microtiter plate assay, reversible flow chromatographic binding assay, an enzyme linked immunosorbent assay, a radioimmunoassay, a hemagglutination assay, a western blot assay, a fluorescence polarization immunoassay, or an indirect immunofluorescence assay.

[00211] Also described herein are methods of detecting an *Anaplasma platys* infection or exposure to *Anaplasma platys* in a subject comprising: (a) obtaining a biological sample from the subject; (b) contacting a purified polypeptide comprising the amino acid sequence of one or more of the following: (i) P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof; (ii) a variant of P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof; (iii) an antigenic fragment of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof; (iv) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof; (v) a variant of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof; (vi) an antigenic fragment of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45; (vii)

SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof; (viii) a variant of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof; (ix) an antigenic fragment of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, or SEQ ID NO: 97; (x) the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof; (xi) a variant of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure

23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof; (xii) an antigenic fragment of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof; (xiii) a combination of one or more of the sequences in (i) – (xii); (xiv) OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof; (xv) a variant of OMP-1X Box

1, OMP-1X Box 2, or a fragment thereof; ((xvi) an antigenic fragment of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof; (xvii) SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof; (xviii) a variant of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof; (xix) an antigenic fragment of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or SEQ ID NO: 9; (xx) SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof; (xxi) a variant of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof; (xxii) an antigenic fragment of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, or SEQ ID NO: 8; (xxiii) the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; (xxiv) a variant of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; (xxv) an antigenic fragment of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to

about position 300 of Figure 22, or a fragment thereof; or (xxvi) a combination of one or more of the sequences in (xiv) – (xxv); with the biological sample under conditions that allow polypeptide/antibody complexes to form; and (c) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that the subject has an *Anaplasma platys* infection or exposure to *Anaplasma platys*. In one aspect, the methods of detecting an *Anaplasma platys* infection or exposure to *Anaplasma platys* in a subject can further comprise contacting the polypeptide/antibody complexes of step (b) with an indicator reagent that generates a measurable signal prior to the performance of step (c).

[00212] In a further aspect, the purified protein can be a fusion protein. For example, and not to be limiting, the purified polypeptide can be fused to an indicator reagent, an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, a transmembrane domain, a protein purification ligand, a heterologous protein, or a combination thereof. In yet a further aspect, the polypeptide/antibody complexes can be detected at about 10 days after exposure or infection of the subject by *Anaplasma platys*.

[00213] Further described herein are methods of detecting *Anaplasma platys* polypeptides in a test sample comprising: (a) contacting one or more antibodies that specifically bind to a *Anaplasma platys* polypeptide with the test sample under conditions that allow polypeptide/antibody complexes to form; wherein the *Anaplasma platys* polypeptide comprises the amino acid sequence of one or more of the following: (i) P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof; (ii) a variant of P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof; (iii) an antigenic fragment of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof; (iv) SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof; (v) a variant of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof; (vi) an antigenic fragment of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43,

SEQ ID NO: 44, or SEQ ID NO: 45; (vii) SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof; (viii) a variant of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof; (ix) an antigenic fragment of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, or SEQ ID NO: 97; (x) the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof; (xi) a variant of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid

sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof; (xii) an antigenic fragment of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof; (xiii) a combination of one or more of the sequences in (i) – (xii);

(xiv) OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof; (xv) a variant of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof; ((xvi) an antigenic fragment of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof; (xvii) SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof; (xviii) a variant of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof; (xix) an antigenic fragment of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or SEQ ID NO: 9; (xx) SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof; (xxi) a variant of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof; (xxii) an antigenic fragment of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, or SEQ ID NO: 8; (xxiii) the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; (xxiv) a variant of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; (xxv) an antigenic fragment of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid

from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; or (xxvi) a combination of one or more of the sequences in (xiv) – (xxv); (b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that an *Anaplasma platys* polypeptide is present in the test sample. For example, and not to be limiting, the one or more antibodies can be monoclonal antibodies, polyclonal antibodies, Fab fragments, Fab' fragments, Fab'-SH fragments, $F(ab')_2$ fragments, Fv fragments, or single chain antibodies. In one aspect, the complexes of step (a) can be contacted with an indicator reagent prior to the performance of step (b).

[00214] In one aspect, the methods of detecting *Anaplasma platys* polypeptides in a test sample can further comprise determining the amount of *Anaplasma platys* polypeptides in the test sample. In yet a further aspect, the one or more antibodies can be attached to a substrate.

[00215] In a further aspect, the methods of detecting *Anaplasma platys* polypeptides in a test sample can further comprise a microtiter plate assay, reversible flow chromatographic binding assay, an enzyme linked immunosorbent assay, a radioimmunoassay, a hemagglutination assay, a western blot assay, a fluorescence polarization immunoassay, or an indirect immunofluorescence assay.

[00216] The methods described herein can be used to detect antibodies or antibody fragments specific for *Anaplasma platys* polypeptides, *Anaplasma platys* polynucleotides, or a combination thereof in a test sample, such as a biological sample, an environmental sample, or a laboratory sample. A test sample can potentially comprise *Anaplasma platys* polynucleotides, *Anaplasma platys* polypeptides, or antibodies specific for *Anaplasma platys*. A biological sample can include, for example, sera, blood, cells, plasma, or tissue from a mammal such as a horse, cat, dog or human. The test sample can be untreated, precipitated, fractionated, separated, diluted, concentrated, or purified.

[00217] Disclosed herein are methods that comprise contacting an *Anaplasma platys* polypeptide with a test sample under conditions that allow a polypeptide/antibody complex, i.e., an immunocomplex, to form. That is, one or more of the polypeptides described herein specifically binds to an antibody specific for *Anaplasma platys* antigens located in the sample. One of skill in the art is familiar with assays and conditions that are used to detect antibody/polypeptide complex binding. The formation of a complex between polypeptides

and anti- *Anaplasma platys* in the sample is detected. In one embodiment of the invention antibody-polypeptide complexes can be detected at about 10, 15, 20, 25, 30 or less days after exposure or infection of the subject by *Anaplasma platys*.

[00218] The antibodies described herein can be used in a method of the diagnosis of *Anaplasma platys* infection by obtaining a test sample from, e.g., a human or animal suspected of having an *Anaplasma platys* infection. Exposure to *Anaplasma platys* can also be detected. Exposure would include the presence of *Anaplasma platys* organisms without clinical symptoms and prior infection with *Anaplasma platys*. The test sample is contacted with antibodies of the invention under conditions enabling the formation of antibody-antigen complexes (i.e., immunocomplexes). The amount of antibody-antigen complexes can be determined by methodology known in the art. A level that is higher than that formed in a control sample indicates an *Anaplasma platys* infection. A control sample is a sample that does not comprise any *Anaplasma platys* polypeptides or antibodies specific for *Anaplasma platys*. In one embodiment of the invention an antibody is specific for *Anaplasma platys* P44 or *Anaplasma platys* OMP-1X antigens only. Alternatively, a polypeptide of the invention can be contacted with a test sample. *Anaplasma platys* antibodies in a positive body sample will form an antigen-antibody complex under suitable conditions. The amount of antibody-antigen complexes can be determined by methods known in the art.

[00219] Also disclosed herein are methods wherein the polypeptide/antibody complex is detected when an indicator reagent, such as an enzyme conjugate, which is bound to the antibody, catalyzes a detectable reaction. Optionally, an indicator reagent comprising a signal generating compound can be applied to the polypeptide/antibody complex under conditions that allow formation of a polypeptide/antibody/indicator complex. The polypeptide/antibody/indicator complex is detected. Optionally, the polypeptide or antibody can be labeled with an indicator reagent prior to the formation of a polypeptide/antibody complex. The method can optionally comprise a positive or negative control.

[00220] Disclosed herein are methods wherein one or more of the antibodies disclosed herein are attached to a solid phase or substrate. A test sample potentially comprising a protein comprising one or more of the polypeptides described herein is added to the substrate. Antibodies that specifically bind to one or more of the polypeptides described

herein are added. The antibodies can be the same antibodies used on the solid phase or can be from a different source or species and can be linked to an indicator reagent, such as an enzyme conjugate. Wash steps can be performed prior to each addition. A chromophore or enzyme substrate can be added and color is allowed to develop. The color reaction can be stopped and the color can be quantified using, for example, a spectrophotometer.

[00221] Also disclosed herein are methods wherein one or more of the antibodies described herein are attached to a solid phase or substrate. A test sample potentially comprising a protein comprising one or more of the polypeptides described herein is added to the substrate. Second anti-species antibodies that specifically bind one or more of the polypeptides described herein are added. These second antibodies can be from a different species than the solid phase antibodies. Third anti-species antibodies can also be added that specifically bind the second antibodies and that do not specifically bind the solid phase antibodies are added. The third antibodies can comprise an indicator reagent such as an enzyme conjugate. Wash steps can be performed prior to each addition. A chromophore or enzyme substrate is added and color is allowed to develop. The color reaction is stopped and the color can be quantified using, for example, a spectrophotometer.

[00222] Disclosed herein are methods of detecting antibodies that specifically bind an *Anaplasma platys* polypeptide or both. The method comprises contacting one or more of the purified polypeptides described herein with a test sample, under conditions that allow polypeptide/antibody complexes to form and detecting polypeptide/antibody complexes. The detection of polypeptide/antibody complexes is an indication that antibodies specific for *Anaplasma platys* are present in the test sample, and the absence of polypeptide/antibody complexes is an indication that antibodies specific for *Anaplasma platys* are not present in the test sample. The complexes can be contacted with an indicator reagent prior to the detection step. The amount of antibody in the test sample can be determined. The purified polypeptide can be attached to a substrate. The purified polypeptide can be a fusion protein wherein the purified polypeptide is fused to an indicator reagent, an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, a transmembrane domain, a protein purification ligand, a heterologous protein, or a combination thereof. The purified polypeptide can be in multimeric form. The method can comprise a microtiter plate assay, reversible flow chromatographic binding assay, an enzyme linked immunosorbent assay, a radioimmunoassay, a hemagglutination assay a

western blot assay, a fluorescence polarization immunoassay, or an indirect immunofluorescence assay.

[00223] Disclosed herein are methods of detecting an *Anaplasma platys* infection and/or exposure to *Anaplasma platys* in a subject. The method can comprise obtaining a biological sample from the subject; contacting a purified polypeptide of the invention with the biological sample under conditions that allow polypeptide/antibody complexes to form; and detecting polypeptide/antibody complexes. The detection of polypeptide/antibody complexes is an indication that the subject has an *Anaplasma platys* infection and/or exposure to *Anaplasma platys*. The absence of polypeptide/antibody complexes is an indication that the mammal has not had an *Anaplasma platys* infection and/or exposure to *Anaplasma platys*. The polypeptide/antibody complexes can be contacted with an indicator reagent that generates a measurable signal prior to the performance of the detection step. The purified polypeptide can be a fusion protein wherein the purified polypeptide is fused to an indicator reagent, an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, a transmembrane domain, a protein purification ligand, a heterologous protein or a combination thereof. The polypeptide/antibody complexes can detected at about 10 days after exposure or infection of subject by *Anaplasma platys*.

[00224] Also described herein are assays that include, but are not limited to those based on competition, direct reaction or sandwich-type assays, including, but not limited to enzyme linked immunosorbent assay (ELISA), western blot, IFA, radioimmunoassay (RIA), hemagglutination (HA), fluorescence polarization immunoassay (FPIA), and microtiter plate assays (any assay done in one or more wells of a microtiter plate). One assay comprises a reversible flow chromatographic binding assay. For example, described herein are assays similar to those described in U.S. Pat. No. 5,726,010.

[00225] Assays can use solid phases or substrates or can be performed by immunoprecipitation or any other methods that do not utilize solid phases. Where a solid phase or substrate is used, one or more of the polypeptides described herein can be directly or indirectly attached to a solid support or a substrate such as a microtiter well, magnetic bead, non-magnetic bead, column, matrix, membrane, fibrous mat composed of synthetic or natural fibers (e.g., glass or cellulose-based materials or thermoplastic polymers, such as, polyethylene, polypropylene, or polyester), sintered structure composed of particulate

materials (e.g., glass or various thermoplastic polymers), or cast membrane film composed of nitrocellulose, nylon, polysulfone or the like (generally synthetic in nature). For example, the substrate can be sintered, fine particles of polyethylene, commonly known as porous polyethylene, for example, 10-15 micron porous polyethylene from Chromex Corporation (Albuquerque, N. Mex.). All substrate materials can be used in suitable shapes, such as films, sheets, or plates, or they can be coated onto or bonded or laminated to appropriate inert carriers, such as paper, glass, plastic films, or fabrics. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like.

[00226] Disclosed herein is an assay format, wherein one or more polypeptides can be coated on a solid phase or substrate. A test sample suspected of containing an anti-*Anaplasma platys* antibody or fragment thereof is incubated with an indicator reagent comprising a signal generating compound conjugated to an antibody or antibody fragment specific for *Anaplasma platys* for a time and under conditions sufficient to form antigen/antibody complexes of either antibodies of the test sample to the polypeptides of the solid phase or the indicator reagent compound conjugated to an antibody specific for *Anaplasma platys* to the polypeptides of the solid phase. The reduction in binding of the indicator reagent conjugated to an anti-*Anaplasma platys* antibody to the solid phase can be quantitatively measured. A measurable reduction in the signal compared to the signal generated from a confirmed negative *Anaplasma platys* test sample indicates the presence of anti-*Anaplasma platys* antibody in the test sample. This type of assay can quantitate the amount of anti-*Anaplasma platys* antibodies in a test sample.

[00227] Disclosed herein is an assay format, wherein one or more of the polypeptides disclosed herein are coated onto a support or substrate. One or more of the polypeptides disclosed herein can be conjugated to an indicator reagent and added to a test sample. This mixture can then be applied to the support or substrate. If *Anaplasma platys* antibodies are present in the test sample they will bind the polypeptide conjugated to an indicator reagent and to the polypeptide immobilized on the support. The polypeptide/antibody/indicator complex can then be detected. This type of assay can quantitate the amount of anti-*Anaplasma platys* antibodies in a test sample.

[00228] Disclosed herein is an assay format, wherein one or more polypeptides disclosed herein are coated onto a support or substrate. The test sample can be applied to the support or substrate and incubated. Unbound components from the sample can be washed away by washing the solid support with a wash solution. If *Anaplasma platys* specific antibodies are present in the test sample, they will bind to the polypeptide coated on the solid phase. This polypeptide/antibody complex can be detected using a second species-specific antibody that is conjugated to an indicator reagent. The polypeptide/antibody/anti-species antibody indicator complex can then be detected. This type of assay can quantitate the amount of anti- *Anaplasma platys* antibodies in a test sample.

[00229] The formation of a polypeptide/antibody complex or a polypeptide/antibody/indicator complex can be detected by radiometric, colormetric, fluorometric, size-separation, or precipitation methods. Optionally, detection of a polypeptide/antibody complex is by the addition of a secondary antibody that is coupled to an indicator reagent comprising a signal generating compound. Indicator reagents comprising signal generating compounds (labels) associated with a polypeptide/antibody complex can be detected using the methods described above and include chromogenic agents, catalysts such as enzyme conjugates fluorescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as dioxetanes, acridiniums, phenanthridiniums, ruthenium, and luminol, radioactive elements, direct visual labels, as well as cofactors, inhibitors, magnetic particles, and the like. Examples of enzyme conjugates include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

[00230] Disclosed herein is an assay format wherein the *Anaplasma platys* polypeptides, polynucleotides, antibodies or combinations thereof can be used in conjunction with Raman spectroscopy. Raman spectroscopy is an analytical technique for chemical and biological analysis due to the wealth of information on molecular structures, surface processes, and interface reactions that can be extracted from experimental data. The Raman technique has been used with gene probe biosensors. U.S. Pat. No. 5,814,516 ('516 patent) discloses a gene probe biosensor comprising a support means, a SERS gene probe having at least one oligonucleotide strand having at least one SERS label, and a SERS active substrate disposed on the support means. The support means has at least one SERS gene probe adsorbed

thereon. Biotargets such as bacterial and viral DNA, RNA and PNA are detected using a SERS gene probe via hybridization to oligonucleotide strands complementary to the SERS gene probe. U.S. Pat. No. 5,814,516 is hereby incorporated by reference in its entirety for it's teaching of the Raman technique.

[00231] The '516 patent does not disclose or suggest operatively connecting a Raman gene probe with an integrated circuit detection system to produce a biochip capable of SERS detection. U.S. Pat. No. 7,267,948 ('948 patent) provides another assay format wherein the *Anaplasma platys* polypeptides, polynucleotides, antibodies or combinations thereof can be used. This '948 patent describes Raman and SERS assay methods and systems including microarrays, biosensors and biochips for the detection of biotargets such as DNA, proteins and pathogens using receptor probes. Receptor probes may include one or more bioreceptors selected from antibodies, DNA, enzymes, tissues, organelles, as well as other receptor probes, and combinations thereof described herein. U.S. Pat. No. 7,267,948 is hereby incorporated by reference in its entirety for it's teaching of the Raman and SERS assay methods and systems.

[00232] Formation of the complex is indicative of the presence of anti- *Anaplasma platys* antibodies in a test sample. Therefore, the methods of the invention can be used to diagnose *Anaplasma platys* infection or exposure in a patient.

[00233] The methods described herein can also indicate the amount or quantity of anti-*Anaplasma platys* antibodies in a test sample. With many indicator reagents, such as enzyme conjugates, the amount of antibody present is proportional to the signal generated. Depending upon the type of test sample, it can be diluted with a suitable buffer reagent, concentrated, or contacted with a solid phase without any manipulation. For example, serum or plasma samples that previously have been diluted, or concentrate specimens such as urine, can be tested in order to determine the presence and/or amount of antibody present.

[00234] The polypeptides and assays described herein can be combined with other polypeptides or assays to detect the presence of *Anaplasma platys* along with other organisms. For example, polypeptides and assays of the invention can be combined with reagents that detect heartworm and/or *Borrelia burgdorferi* and/or *Anaplasma phagocytophilium* and/or *Ehrlichia canis*.

[00235] Also disclosed herein are methods of detecting an *Anaplasma* infection or exposure to *Anaplasma* in a subject. These methods can be used as an initial or final method to identify the presence of one or more species of *Anaplasma*. *Anaplasma* is a genus of rickettsiales bacteria. Anaplasmas can reside in host red blood cells and lead to the disease anaplasmosis.

Anaplasmas can require intermediate tick hosts for maturation, and flies may act as mechanical vectors. Species of *Anaplasma* include, but are not limited to, *Anaplasma marginale*, *Anaplasma centrale*, *Anaplasma mesaeterum*, *Anaplasma ovis*, and *Anaplasma platys*.

[00236] Disclosed herein are methods of detecting an *Anaplasma* infection or exposure to *Anaplasma* in a subject comprising: (a) obtaining a biological sample from the subject; (b) contacting a purified polypeptide encoded by one or more of the nucleotides of the following: SEQ ID NOs: 124-132 or a combination of one or more of the sequences of SEQ ID NOs: 124-132; with the biological sample under conditions that allow polypeptide complexes to form; and (c) detecting polypeptide complexes; wherein the detection of polypeptide complexes is an indication that the subject has an *Anaplasma* infection or exposure to *Anaplasma*. In addition, the methods can further comprise contacting the polypeptide complexes with an antibody that recognizes the polypeptide or polypeptide complex.

[00237] Also disclosed are methods of detecting the presence of *Anaplasma* in a sample by contacting said sample with a DNA probe or primer constructed to correspond to an *Anaplasma* P44 nucleotide sequence, characterized in that the probe or primer comprises one or more of the nucleotides of SEQ ID NOs: 124-132.

[00238] Also disclosed are methods of detecting the presence of *Anaplasma* in a sample by contacting said sample with a DNA probe or primer constructed to correspond to an *Anaplasma* P44 nucleotide sequence, characterized in that the probe or primer comprises one or more nucleotides capable of hybridizing to one or more of the nucleotides of SEQ ID NOs: 124-132.

[00239] A method for detecting *Anaplasma* in a sample obtained from a subject, comprising (a) providing a primer set comprising: (i) one or more forward primers capable of hybridizing to or amplifying: one or more of the nucleotides of SEQ ID NOs: 124-132

and (ii) one or more forward primers capable of hybridizing to or amplifying: one or more of the nucleotides of SEQ ID NOs: 124-132; (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and (c) determining the length or sequence of the PCR products of step (b), wherein the presence of a PCR product having a length or sequence which corresponds to the length or sequence, respectively, of that region of the *Anaplasma* nucleotide sequence which is located between the regions to which the one or more forward primers and the one or more reverse primers bind is indicative of the presence of *Anaplasma* in the sample.

[00240] Also disclosed herein are isolated or purified polynucleotides comprising the sequence of one or more of the polynucleotide sequences of SEQ ID NOs: 124-132, or a fragment thereof. Also disclosed herein are isolated or purified polynucleotides comprising 80%, 85%, 90%, 95%, or 100% sequence identity to the sequence of one or more of the polynucleotide sequences of SEQ ID NOs: 124-132, or a fragment thereof.

[00241] Also disclosed are vectors for transformation of a host cell, said vector comprising the sequence of one or more of the polynucleotide sequences of SEQ ID NOs: 124-132, or a fragment thereof.

[00242] Also disclosed herein are isolated or purified polypeptides encoded by a polynucleotide sequences wherein the polynucleotide sequence comprises the sequence of one or more of the polynucleotide sequences of SEQ ID NOs: 124-132, or a fragment thereof. Also disclosed herein are isolated or purified polypeptides encoded by a polynucleotide sequences wherein the polynucleotide sequence comprises 80%, 85%, 90%, 95%, or 100% sequence identity to the sequence of one or more of the polynucleotide sequences of SEQ ID NOs: 124-132, or a fragment thereof.

## EXAMPLES

### EXAMPLE 1

### MATERIALS AND METHODS

[00243] *A. platys-infected dogs*. Dogs that were naturally infected with *A. platys* were identified in Lara, Venezuela, in 2007 by observation of bacterial inclusions (morulae) in

platelets from blood smears, and cases were confirmed by PCR and sequencing using primer pairs specific for *A. platys* 16S rRNA (EP1-EP3 and EP2-EP3).[29] Naturally infected dogs in Taichung, Taiwan and the Democratic Republic of Congo were identified and confirmed by PCR using primer pair EPLAT5-EPLAT.[42]

[00244] *Cloning of p44 expression locus from A. platys.* DNA samples from three dogs from Venezuela and one dog from Taiwan were used as templates. By aligning the *p44/msp2* expression loci from *A. phagocytophilum* and *A. marginale*, several degenerate primers were designed for conserved regions of the locus (Figure 1, Table 1). Using the first and the second primer pairs F1-R1 and F1-R2, (hemi-) nested touchdown PCR[52] was used to amplify the *tr1* and *omp-1X* gene sequences from *A. platys*. In order to avoid truncating *p44* pseudogenes in the *A. platys* genome, primer F3 was designed to be upstream of the predicted *p44* open reading frame. *p44ES* sequences were amplified by nested touchdown PCR using primer pairs F2-R3 and F3-R4. Amplification was performed as previously described.[68] The amplified DNA fragments were cloned using the TA cloning kit (Invitrogen, Carlsbad, CA) and sequenced with M13 forward or M13 reverse sequencing primers. All sequencing data were assembled using the SeqMan program (DNASTAR Inc., Madison, WI). To confirm the assembly the entire locus was amplified using primers F1 and R5 (Table 1).

[00245] *Phylogenetic analysis.* The deduced amino acid sequences for Tr1, OMP-1/OMP-1X, and P44ES from *A. marginale*, *A. phagocytophilum,* and *A. platys* were aligned using the MegAlign program (DNASTAR Inc.) by the Clustal W method.

[00246] *Protein structure analysis using bioinformatics tools***.** SignalP 3.0 server trained on Gram-negative bacteria (http://www.cbs.dtu.dk/services/SignalP/) was used for signal peptide sequence analysis. The secondary structures of P44 and OMP-1X were predicted by PRED-TMBB[4] and hydrophobicity analysis and the hydrophobic moment profile method, as previously described.[30, 35] The antigenic index and surface probability were determined using the Protean program (DNASTAR Inc.).

[00247] *ELISA analysis of OMP-1X-specific peptide.* The OMP-1X peptide from *A. platys* was synthesized at Biomatik (Wilmington, DE). The purity of the peptide was greater than 98%, as assessed by high-performance liquid chromatography. The wells of a 96-well microtiter plate were coated with 200 ng peptide/well and the ELISA was performed as previously described.[60] Samples were from three dogs that were PCR-positive for *A. platys*

(TW 431, TW 270, and TW 210), and three dogs that were both PCR-negative for *A. platys* and antigen dot blot–negative for *A. phagocytophilum* (E05-290, E10-0062, and E10-0075). In addition, horse anti–*A. phagocytophilum* positive sera (EQ002, EQ006, and E09-0011)[65, 71] were used to confirm the absence of OMP-1X peptide antigen cross-reactivity with anti-*A. phagocytophilum* antibodies. The horseradish peroxidase substrate 2,2'-azido-di-(3-ethyl)-benzthiazoline-6-sulfonic acid (Sigma, St. Louis, MO) in 70 mM citrate buffer (pH 4.2) applied, and absorbance values at 415 and 492 nm were measured in an ELISA plate reader (Molecular Devices, Sunnyvale, CA) as previously described.[68] Results were presented as optical density at 415 nm minus that at 492 nm ($OD_{415} - OD_{492}$), and the cutoff for a positive reaction was set at greater than the mean $OD_{415} - OD_{492}$ + 3 SD for the negative control samples (OD > 0.165). The assay was repeated at least three times.

[00248] *Nucleotide sequence accession number.* The *A. platys tr1-omp-1X-p44ES* sequences from two naturally infected dogs from Venezuela were assembled and deposited at GenBank under accession numbers GQ868750 and GU357491. Additional *p44ES* and *p44* sequences were deposited at GenBank under accession numbers GU357492, GU357493, GU357494, GU357495, GU357496, GU357497, and HQ738571.

RESULTS

[00249] *A. platys tr1-omp1-p44ES cluster sequencing and assembly.* Three degenerate primers and one primer at a highly conserved region of *tr1* upstream region were designed based on conserved regions of the aligned *tr1-omp1X-omp1N-p44* cluster of *A. phagocytophilum* and YP_154239 (hypothetical protein AM1138; transcriptional regulator)-YP_154240 (outer membrane protein 1; outer membrane protein 4)-YP_154241 (*msp2* operon associated gene 3; outer membrane protein 3)-YP_154243 (*msp2* operon associated gene 2)-YP_154244 (*msp2* operon associated gene 1)-YP_154245 (*msp2*) cluster of *A. marginale* (Figure 1, Table 1). The first touchdown PCR was designed to amplify the entire fragment using primers F1 and R1. The PCR products from the two dog DNA specimens were then used as templates for hemi-nested touchdown PCRs using primers F1 and R2 (Figure 1, Table 1). As a result, a single band of about 2,100 bp was amplified (fragment A). The PCR products were TA cloned and sequenced. The sequence results showed this fragment contained *A. platys tr1* and *omp-1X*. The downstream region of fragment A was amplified by nested touchdown PCR, using the PCR products obtained with primers F1 and R1 as templates, with primer F2 designed based on the fragment A sequence and primer R3

designed based on *p44* (*msp2*) sequences conserved between *A. phagocytophilum* and *A. marginale*. As a result, a single band of approximately 1,100 bp was amplified (fragment B). The PCR products were TA cloned and sequenced. The sequence results showed that this fragment contained a partial *A. platys p44* sequence. To amplify *A. platys* full-length *p44*, primer F3 was designed based on fragment B sequence and primer R4 was designed based on the conserved region of *valS* found downstream of *p44* (*msp2*) in both *A. phagocytophilum* and *A. marginale*. Further touchdown PCR was done using primers F1 and R4. The PCR products were then used as templates for hemi-nested touchdown PCRs using primers F3 and R4. As a result, a single band of about 1,700 bp was amplified (fragment C). The PCR products were TA cloned and sequenced. The sequence results showed this fragment contained full length *A. platys p44* sequences. The final assembled sequences 3,957 bp from dogs 1 and 2, respectively contained the entire *A. platys p44ES* locus, and the G+C content was 47.46~47.51 %.

[00250] The final assembled sequence of 3957 bp from Venezuelan dogs 1 and 2 contained the entire *A. platys p44ES* locus. The sequence average coverage of the entire locus was 8.3 fold (5 to 15 fold). To confirm the assembly was from a complete genomic locus, primer R5 was designed to be downstream of the predicted *p44* open reading frame and one more touchdown PCR was conducted using primers F1 and R5. A single band, approximately 3.9 kb was amplified (fragment D), indicating the fragment containing the entire locus was amplified from the blood of dog 2 (Figure 1C). The G+C content was determined to be 47.46% to 47.51%. The synteny among the entire outer membrane protein gene clusters of *A. platys* and the two previously sequenced *Anaplasma* species was analyzed using the Artemis Comparison Tool[14] The *tr1* sequences were conserved among the three *Anaplasma* species (Figure 2). The 5′ and 3′ regions of *p44ES* were conserved between *A. platys* and *A. phagocytophilum*, but not between *A. platys* and *A. marginale* (Figure 2).

[00251] *A. platys Tr1 structure.* Three similar (97.8% to 99.5%) *A. platys tr1* sequences were obtained from two dogs from Venezuela and one dog from Taiwan. The predicted molecular mass of *A. platys* Tr1 was 21.0 to 21.1 kDa with an isoelectric point of 5.50 to 5.80 (Table 2). Tr1 was not predicted to have a signal peptide, thus was a cytoplasmic protein, as analyzed by SignalP 3.0. Tr1 was predicted to contain a putative N-terminal helix-turn-helix DNA-binding domain based on the analysis of the NCBI conserved domain database, suggesting that it was a transcriptional regulator. The amino acid sequence identity between

*A. platys* Tr1 and *A. phagocytophilum* Tr1 (YP_505749) was 84.8% to 86.4%, and that between *A. platys* Tr1 and *A. marginale* Tr (YP_154239) was 73.1% to 74.1%.

[00252] *A. platys OMP-1X structure.* Three nearly identical (99.1%) *A. platys omp-1X* sequences were obtained from two dogs from Venezuela and one dog from Taiwan. Using SignalP 3.0 server, OMP-1X was predicted to have a signal peptide with a cleavage site between positions 23 and 24. The predicted molecular mass of mature *A. platys* OMP-1X was 31.9 kDa with an isoelectric point of 7.27 to 7.92 (Table 2). The secondary structure of OMP-1X was then examined, using PRED-TMBB.[4] The discrimination value of the OMP-1X amino acid sequence was 2.907, which is below the threshold value of 2.965, making OMP-1X likely to be a β-barrel protein localized to the outer membrane. Hydrophobicity analysis and the hydrophobic moment profile program developed for the porin structure prediction,[35] predicted 14 β-strands in OMP-1X. The protein sequences most closely related to *A. platys* OMP-1X were *A. phagocytophilum* OMP-1X (YP_505750; 45.9%-46.3% identity) and *A. marginale* OMP-1 (YP_154240; 39.8% identity). A phylogenetic analysis showed that OMP-1X homologs in *Anaplasma* spp. formed a cluster that was distinct from the cluster of most closely related OMP-1X homologs in each *Ehrlichia* spp. (Figure 3).

[00253] *A. platys P44ES structure.* Four *P44ES* sequences (GQ868750, GU357491, GU357492, and GU357493) were obtained from three dogs from Venezuela. Using SignalP 3.0 sever P44ES was predicted to have a putative signal peptide with a cleavage site between positions 21 and 22. The molecular mass of the mature P44ES protein was predicted to be 41.2 to 41.4 kDa with an isoelectric point of 5.30 to 5.72 (Table 2). By PRED-TMBB[4] analysis, the discrimination value of the P44 amino acid sequence was 2.920 which was below the threshold value of 2.965, making P44 likely to be a β-barrel protein localized to the outer membrane. Hydrophobicity analysis and the hydrophobic moment profile predicted 16 β-strands in P44. Alignment of total nine *A. platys* P44 sequences (the four P44 full-length proteins from dogs 1, 2, and 3, and the five partial P44 sequences obtained from dogs 1, 2 and Taiwan) using HVF and HVR primers, revealed a single central hypervariable region (aa position 193-247) of approximately 55 amino acid residues, and N-terminal and C-terminal conserved regions of approximately 192 and 159 amino acid residues, respectively. The conserved amino acids C, C, W, and A from the P44 hypervariable region of *A. phagocytophilum* P44[41] were also detected in the hypervariable region of *A. platys* P44. The C terminus of *A. platys* P44 ends with phenylalanine, as does the C terminus of *A.*

*phagocytophilum* P44.[30] The amino acid sequence identity between *A. platys* P44ES and *A. phagocytophilum* P44-18ES (YP_505752) was 55.0% to 56.9%, and that between *A. platys* P44ES and *A. marginale* Msp2 (YP_154245) was 41.5% to 42.1%. Phylogenetic analysis placed full-length *A. platys p44s* between *A. phagocytophilum p44*s and *A. marginale msp2*s (Figure 4). The sequence identities of the conserved N-terminal 192 amino acids and the conserved C-terminal 159 amino acids of *A. platys* and *A. phagocytophilum* P44s were 57.3% and 66.7%, respectively.

[00254] Primer pairs (HVF and HVR, Table 1) designed based on *A. platys p44* conserved region amplified only *A. platys* DNA, but not *A. phagocytophilum* and *A. marginale* DNA. Alignment of a total of nine *A. platys* P44 hypervariable regions and flanking conserved regions with P44/Msp2 sequences among *A. phagocytophilum* P44s and *A. marginale* Msp2s revealed several *A. platys*-specific sequences: TGTAAGSDVDYVSKF (SEQ ID NO: 92; aa position 23-37), TRVEWKAE (SEQ ID NO: 93; aa position 78-85), AAEIVKFAEAVGTSAK (SEQ ID NO: 94; aa position 174-189), SWKCTQTG (SEQ ID NO: 95; aa position 207-214), AAKAEDLS (SEQ ID NO: 96; aa position 248-255) and ATTNKTKEF (SEQ ID NO: 97; aa position 378-386). These *A. platys*-specific *p44* regions were utilized as serologic test antigens to distinguish from *A. phagocytophilum* or *A. marginale* infections.

[00255] **Table 1**. Primers used for PCR amplification of A. platys P44ES cluster

| Primer | Sequence | Note |
|---|---|---|
| F1 | 5′-ATTATGTATGATTTATCCTAAGTTATCTGAG-3′ (SEQ ID NO: 82) | *tr1/tr* highly conserved upstream region |
| F2 | 5-GGGATATCGGCGTTGATAGGG-3′ (SEQ ID NO: 83) | *A. platys omp-1X* downstream genomic walking |
| F3 | 5′-GGTTTGTGTTGCTGGTGATTGGAGG-3′ (SEQ ID NO: 84) | *A. platys p44* upstream genomic walking |
| R1 | 5′-GCAAACCTAACACCMAAYTCMCCACC-3′ (SEQ ID NO: 85) | *p44ES/Msp2* C-terminal highly conserved region |
| R2 | 5′-TATACTAAAAAAGAATTAAGTCAAGAG-3′ (SEQ ID NO: 86) | Conserved intergenetic region between *omp-1X /omp1* and *omp-1N/opag3* |
| R3 | 5′-ATGGTAGAAASCCCCAGCAAA-3′ (SEQ ID NO: 87) | *p44ES/Msp2* N-terminal conserved region |
| R4 | 5′-CACGTNTTTAGTTACTGCCA-3′ (SEQ ID NO: 88) | *p44ES/Msp2ES* from downstream conserved *valS* gene |

| | | |
|---|---|---|
| R5 | 5′-GTACTAGTCAGCGCCACTAACATCAA-3′ (SEQ ID NO: 89) | *p44ES/Msp2ES* downstream region for complete locus confirmation |
| HVF | 5′-GAAGAATACGAAAGCGGCGG-3′ (SEQ ID NO: 90) | *A. platys* P44 hypervariable cloning region |
| HVR | 5′-TACTTAGGTCTTCCGCTTTCGC-3′ (SEQ ID NO: 91) | *A. platys* P44 hypervariable cloning region |

[00256] *ELISA analysis of OMP-1X.* When the Clustal W method was used to compare *A. platys* OMP-1X to its phylogenetically closest OMP-1 homologs-*A. phagocytophilum* OMP-1X (YP_505750), *A. marginale* OMP1 (YP_154240), *E. canis* P30-19 (AAK28680), *E. ruminantium* Map1-related protein (YP_180721), *E. ewingii* OMP-1-1 (ABO36240), and *E. chaffeensis* OMP-1M (YP_507903), we identified a unique region in the *A. platys* OMP-1X amino acid sequence. This sequence, AVQEKKPPEA (SEQ ID NO: 98), is within the 2nd external loop from the N-terminus based on the hydrophobicity analysis and the hydrophobic moment profile program. The sequence is predicted to be highly antigenic and surface exposed by Protean program may aid in differential serodiagnosis (Figure 14). The *A. platys* OMP-1X peptide was synthesized and its reactivity to known infected dog sera was tested by ELISA. Three *A. platys* PCR–positive dog sera reacted with the synthesized OMP-1X peptide antigen. Sera from *A. platys* PCR−negative dogs and horse anti–*A. phagocytophilum* serum did not react with the OMP-1X peptide antigen (Figure 15), suggesting that this antigen can be used for species-specific serodiagnosis of *A. platys*.

[00257] **Table 2**. Properties of A. platys p44ES cluster

| Protein | Upstream intergenic space (bp) | Gene Length (bp) | Protein Size (amino acids)[a] | Signal peptide (amino acids)[b] | Molecular mass[a] (Da) | Isoelectric point | Protein | Upstream intergenic space (bp) |
|---|---|---|---|---|---|---|---|---|
| Tr1 | NA[c] | 558 | 185 | NA | 21010.6-20952.6[d] | 5.50-5.80[d] | Tr1 | NA[c] |
| OMP-1X | 306 | 933 | 301 | 23 | 31885.0-31942.1[d] | 7.27-7.92[d] | OMP-1X | 306 |
| P44ES | 682 | 1221 | 380-386[d] | 21 | 41167.3-41359.5[d] | 5.30-5.72[d] | P44ES | 682 |

[a] Mature protein.
[b] Predicted cleavage site.
[c] Not applicable.
[d] Range among strains.

DISCUSSION

[00258] In the present study, the entire 4 kb *A. platys p44ES* locus, containing *tr1*, *omp-1X* and *p44* genes, was sequenced, providing new insight into the *p44* expression locus and the major surface antigen of *A. platys*. From each infected dog different *p44ES* sequences were detected, showing mixed P44 allele population of *A. platys* is present in the dog blood in a given time point similar to *A. phagocytophilum p44* expression in humans and horses[38, 39, 65] or *A. marginale msp2* in the blood of cattle.[18, 21, 47] In addition three more different hypervariable regions were detected in partial *A. platys p44* gene sequences, suggesting the *p44*-expression locus of *A. platys* is also the site of active recombination, and multiple *p44* donor sequences also exist in the *A. platys* genome.

[00259] The synteny analysis suggested that the major outer membrane expression locus existed in a common ancestor of the three *Anaplasma* species in existence today. Furthermore, the locus appears to have diverged primarily by duplicating *omp-1*-like sequences between *tr1* and *p44/msp2ES; A. marginale, A. phagocytophilum,* and *A. platys* have 4, 2, and 1 *omp-1*-like sequences, respectively.

[00260] Three species of *Anaplasma* infect different host cells, namely neutrophils, erythrocytes, and platelets. The comparative study of between *A. phagocytophilum, A. marginale,* and *A. platys* P44/Msp2s, and OMP-1 homologs provided a new window of opportunity to investigate different *Anaplasma* host cell tropism.

[00261] Tr1, a putative transcription factor, is more highly expressed in tick cells infected with *A. phagocytophilum* than in human leukemic HL-60 cells infected with *A. phagocytophilum*, which suggested that Tr1 may regulate genes involved in the bacterial infection cycle in ticks.[44, 64] In contrast, Tr is expressed similarly in bovine red blood cells and IDE8 tick cell cultures infected with *A. marginale*.[5]

[00262] In *A. phagocytophilum tr1,* two *omp-1s* and *p44E* were co-expressed.[39] In the cattle blood, the aforementioned and related genes were co-expressed with the exception of the third msp2-associated genes.[48] Omp-1s are major surface antigens of *Ehrlichia* species, also has a role in *A. platys* infection cycle. OMP-1 homologous proteins are major surface antigens in *Ehrlichia* species,[23, 46, 57, 62, 66] and OMP-1X functions similarly in the *A. platys* infection cycle. *A. platys* OMP-1X is predicted to have a β-barrel structure similar to that of *E. chaffeensis* P28 and OMP-1F,[37] and is thus a porin.

[00263] The fact that *A. phagocytophilum* and *A. marginale* P44/Msp2 transcripts were distinct between mammals and ticks advocates multiple physiological adaptations between different host environments.[44, 53, 71] Furthermore, *A. phagocytophilum p44* gene conversion in mammalian hosts suggested its role in antigenic variation.[8, 19, 38, 65] In cattle, *A. marginale* MSP2s allows antigenic variation for persistent infection.[6, 11, 47] *A. platys* P44, therefore, plays an important role in determining persistent or cyclical rickettsemia.

[00264] It is not known whether *A. platys p44ES* undergoes nonsegmental gene conversion (as in *A. phagocytophilum* to generate identical P44s from a large number of donor loci) or segmental gene conversion (as in *A. marginale* to generate mosaic Msp2ES from a small number of donor loci).[39, 47] P44 has a role in the interaction between *A. phagocytophilum* and host cells.[36, 49, 64] The P44 of *A. phagocytophilum* is the major surface antigen useful for serologic diagnosis of human granulocytic anaplasmosis, and has a role in the interaction between *A. phagocytophilum* and host cells. P44s also elicits a porin activity for passive diffusion of hydrophilic solutes.

[00265] Based on the present study, recombinant or peptide–based OMP-1X and P44 antigen can be prepared for testing the applicability of *A. platys* serodiagnosis. P44 can also serve as a specific and sensitive target for PCR diagnosis for human granulocytic anaplasmosis, and thus can be tested for *A. platys* infection or exposure.

[00266] *A. phagocytophilum* is known to infect dogs in regions where the *Ixodes* tick is endemic.[2, 24, 50, 51] *A. platys* inclusions in the platelets of a naturally infected dog cross-reacted with mouse anti-*A. phagocytophilum* serum.[32] It was important, therefore, to develop a method for distinguishing *A. platys* infection from *A. phagocytophilum* infection. Since the *p44* primer pair: HVF and HVR described herein, is specific to *A. platys,* it was expected to be useful for species-specific PCR diagnosis. P44 of *A. phagocytophilum* is the major surface antigen used for serologic diagnosis of human granulocytic anaplasmosis.[1, 16, 27, 31, 70] In the present study, several *A. platys*-specific amino acid sequences were identified within P44 proteins that can be used as serologic test antigens to provide differential diagnosis from other *Anaplasma* species infection. Additionally, *Ehrlichia* OMP-1/P28/P30/MAP families are immunodominant major outer membrane proteins useful for serodiagnosis.[45, 62, 63, 68] The alignment results showed a distinct fragment (~20 amino acids) in *A. platys* Omp-1X that was not observed in the closest homologs from *Anaplasma* and *Ehrlichia* spp. Furthermore, this region was identical in *A. platys* samples from the geographically separated regions of

Venezuela and Taiwan. This specific OMP-1X peptide antigen did not cross-react with anti-*A. phagocytophilum* serum, and therefore can be suitable for species-specific differential serodiagnosis of *A. platys*.

[00267] Since the only available source of *A. platys* DNA was a small amount of DNA purified from infected dog blood specimens, touchdown PCR was employed to amplify the available canine DNA. Incorrect base pairings resulting from amplification were minimized by using a high-fidelity Taq polymerase

EXAMPLE 2

[00268] *A. platys expression locus analysis.* DNA specimens from three dogs naturally infected with *A. platys* at Lara, Venezuela in 2007 were used as the template for the amplification and sequencing process. *A. platys* infection of the dog was confirmed by PCR and sequencing of the 16S rRNA of *A. platys* as well as by observation of bacterial inclusions (morulae) in platelets in the blood smear. By aligning *A. phagocytophilum* and *A. marginale p44/msp2* expression loci, several degenerate primers were designed based upon conserved regions (Figure 1, Table 1). Using the first primer pair F1 and R1, and the second primer pair F1 and R2, (hemi-) nested touchdown PCR amplified the *A. platys tr1* and *omp-1X* sequences. In order to avoid truncated *p44* pseudogenes in *A. platys* genome, primer F3 for the 5' upstream of the predicted *p44* ORF was designed. *p44ES* sequences were amplified by nested touchdown PCR with primer pairs F2 and R3, and F3 and R4. Amplification was performed as previously described. The amplified DNA fragments were cloned using a TA cloning kit (Invitrogen, Carlsbad, CA) and sequenced with M13 Forward or M13 Reverse sequencing primers. All sequencing data was assembled using SeqMan program of DNASTAR software (DNASTAR Inc., Madison, WI). The deduced amino acid sequences of *A. marginale*, *A. phagocytophilum,* and *A. platys tr1*, *Omp-1X* and *p44ES* were aligned using the MegAlign program of DNASTAR software by the Clustal W method.

[00269] *P44 secondary structure prediction.* The P44 secondary structure was predicted by hydrophobicity and the hydrophobic moment profile method as previously described. Antigenic Index and surface probability were examined by Protean program of DNASTAR.

[00270] All patents, patent applications, and other scientific or technical writings referred to anywhere herein are incorporated by reference in their entirety. The invention illustratively described herein suitably can be practiced in the absence of any element or elements,

limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" can be replaced with either of the other two terms, while retaining their ordinary meanings. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by embodiments, optional features, modification and variation of the concepts herein disclosed can be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

[00271] In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

[00272] Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

[00273] Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided herein may be different from the actual publication dates, which can require independent confirmation.

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## CLAIMS

What is claimed is:

1. A method for detecting *Anaplasma platys* in a sample obtained from a subject, comprising (a) providing a primer set comprising: (i) one or more forward primers comprising the sequence of: SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO:84, or SEQ ID NO: 90 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO:88, SEQ ID NO: 89 or SEQ ID NO: 91; (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and (c) determining the length or sequence of the PCR products of step (b), wherein the presence of a PCR product having a length or sequence which corresponds to the length or sequence, respectively, of that region of the *Anaplasma platys* p44 gene which is located between the regions to which the one or more forward primers and the one or more reverse primers bind is indicative of the presence of *Anaplasma platys* in the sample.

2. The method of claim 1 wherein the one or more forward or reverse primers are from 15 to 35 nucleotides in length.

3. The method of claim 1 wherein the forward primer and the reverse primer, respectively, comprise SEQ ID NO: 90 and SEQ ID NO: 91.

4. A primer set for detecting *Anaplasma platys* in a sample, said primer set comprising: (a) one or more forward primers comprising the sequence of: SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO:84, or SEQ ID NO: 90 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO:88, SEQ ID NO: 89 or SEQ ID NO: 91.

5. The primer set of claim 4 wherein the one or more forward or reverse primers are from 15 to 35 nucleotides in length.

6. The primer set of claim 4 wherein the forward primer and the reverse primer, respectively, comprise SEQ ID NO: 90 and SEQ ID NO: 91.

7. A method for detecting *Anaplasma platys* in a sample obtained from a subject, comprising (a) providing a primer set comprising: (i) one or more forward primers comprising the sequence of: SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO:56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO:57, SEQ ID NO: 59, SEQ ID NO: 61, or SEQ ID NO: 63; (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and (c) determining the length or sequence of the PCR products of step (b), wherein the presence of a PCR product having a length or sequence which corresponds to the length or sequence, respectively, of that region of the *Anaplasma platys* p44 gene which is located between the regions to which the one or more forward primers and the one or more reverse primers bind is indicative of the presence of *Anaplasma platys* in the sample.

8. The method of claim 7 wherein the one or more forward or reverse primers are from 15 to 35 nucleotides in length.

9. The method of claim 7 wherein a forward primer and a reverse primer, respectively, comprise one or more of the following pairs of sequences: PAIR 1: SEQ ID NO: 52 and SEQ ID NO: 53; PAIR 2: SEQ ID NO: 54 and SEQ ID NO: 55; PAIR 3: SEQ ID NO: 56 and SEQ ID NO: 57; PAIR 4: SEQ ID NO: 58 and SEQ ID NO: 59; PAIR 5: SEQ ID NO: 60 and SEQ ID NO: 61; or PAIR 6: SEQ ID NO: 62 and SEQ ID NO: 63.

10. A primer set for detecting *Anaplasma platys* in a sample, said primer set comprising: (a) one or more forward primers comprising the sequence of: SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO:84, or SEQ ID NO: 90 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO:88, SEQ ID NO: 89 or SEQ ID NO: 91.

11. The primer set of claim 10 wherein the one or more forward or reverse primers are from 15 to 35 nucleotides in length.

12. A method for detecting *Anaplasma platys* in a sample obtained from a subject, comprising (a) providing a primer set comprising: (i) one or more forward primers comprising the sequence of: SEQ ID NO: 64, SEQ ID NO: 18,or SEQ ID NO: 20 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 65, SEQ ID

NO: 19 , or SEQ ID NO: 21; (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and (c) determining the length or sequence of the PCR products of step (b), wherein the presence of a PCR product having a length or sequence which corresponds to the length or sequence, respectively, of that region of the *Anaplasma platys* OMP-1X gene which is located between the regions to which the one or more forward primers and the one or more reverse primers bind is indicative of the presence of *Anaplasma platys* in the sample.

13. The method of claim 12 wherein the one or more forward or reverse primers are from 15 to 35 nucleotides in length.

14. The method of claim 12 wherein a forward primer and a reverse primer, respectively, comprise one or more of the following pairs of sequences: PAIR 1: SEQ ID NO: 64 and SEQ ID NO: 65; PAIR 2: SEQ ID NO: 18 and SEQ ID NO: 19; or PAIR 3: SEQ ID NO: 20 and SEQ ID NO: 21.

15. A primer set for detecting *Anaplasma platys* in a sample, said primer set comprising: (a) one or more forward primers comprising the sequence of: SEQ ID NO: 64, SEQ ID NO: 18, or SEQ ID NO: 20 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 65, SEQ ID NO: 19, or SEQ ID NO: 21.

16. The primer set of claim 15 wherein the one or more forward or reverse primers are from 15 to 35 nucleotides in length.

17. An isolated polynucleotide encoding an outer membrane protein of *Anaplasma platys*, or a fragment thereof, wherein the outer membrane protein is P44 or OMP-1X protein.

18. The isolated polynucleotide of claim 17, wherein said outer membrane protein of *Anaplasma platys* is the P44 protein.

19. The isolated polynucleotide of any of claims 17-18, wherein the polynucleotide comprises SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, or a fragment thereof.

20. The isolated polynucleotide of any of claims 17-19, wherein the isolated polynucleotide is capable of encoding SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof.

21. The isolated polynucleotide of claim 17, wherein said outer membrane protein of *Anaplasma platys* is the OMP-1X protein.

22. The isolated polynucleotide of any of claims 17 and 21, wherein the isolated polynucleotide is capable of encoding SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof.

23. The isolated polynucleotide of any of claims 17 and 21-22, wherein the polynucleotide comprises SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or a fragment thereof.

24. The isolated polynucleotide of claim 17, wherein the polynucleotide encodes P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof.

25. The isolated polynucleotide of claim 24, wherein the isolated polynucleotide is capable of encoding SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof.

26. The isolated polynucleotide of claim 24, wherein the isolated polynucleotide is capable of encoding the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about

position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19 the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, a combination or a fragment thereof.

27. The isolated polynucleotide of claim 24, wherein the polynucleotide comprises SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, or SEQ ID NO: 51.

28. The isolated polynucleotide of claim 17, wherein the polynucleotide encodes OMP-1X Box 1, OMP-1X Box 2, OMP-1X Box 1 and OMP-1X Box 2, or a fragment thereof.

29. The isolated polynucleotide of claim 28, wherein the polynucleotide encodes SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof.

30. The isolated polynucleotide of claim 28, wherein the polynucleotide encodes the amino acid sequence comprising the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the

amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof.

31. The isolated polynucleotide of claim 28, wherein the polynucleotide comprises SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, a combination of SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, or a fragment thereof.

32. An isolated polynucleotide encoding a variant of an outer membrane protein of *Anaplasma platys*, or a fragment thereof, wherein the outer membrane protein is P44 or OMP-1X protein.

33. The isolated polynucleotide of Claim 32, wherein said variant has at least 95% identity to SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, the amino acid from about position 66 to about

position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof.

34. The isolated polynucleotide of Claim 33, and wherein said variant is immunoreactive with at least one antibody that binds to P44 protein, P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, OMP-1X protein, OMP-1X Box 1, OMP-1X Box 2, OMP-1X Box 1 and OMP-1X Box 2, or a fragment thereof.

35. An isolated polynucleotide, wherein said polynucleotide comprises SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21.

36. A vector for transformation of a host cell, said vector comprising an isolated polynucleotide that encodes an outer membrane protein of *Anaplasma platys*, a variant of said outer membrane protein, or an immunogenic fragment of said outer membrane protein, wherein said outer membrane protein is the P44 protein, P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, OMP-1X protein, OMP-1X Box 1, OMP-1X Box 2, OMP-1X Box 1 and OMP-1X Box 2, or a fragment thereof.

37. The vector of claim 36, wherein the vector comprising an isolated polynucleotide comprises the nucleotide sequence of any of claims 18-34.

38. The vector of any of claims 36-37, wherein said vector is used in a process for making the corresponding outer membrane protein of *Anaplasma platys*, a variant of said outer membrane protein, or an immunogenic fragment of said outer membrane protein, said process comprising transfecting host cells with said vector and inducing expression of the outer membrane protein or the variant or immunogenic fragment thereof in the host cells.

39. A host cell comprising the expression vector of any of claims 36-38.

40. An isolated or purified polypeptide comprising a sequence chosen from the following: a *Anaplasma platys* P44 protein, a variant of the *Anaplasma platys* P44 protein, an antigenic fragment of the *Anaplasma platys* P44 protein, wherein the *Anaplasma platys* P44 protein comprises or consists of:

    a. P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

    b. a variant of P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

    c. an antigenic fragment of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

    d. SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

    e. a variant of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

f. an antigenic fragment of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45;

g. SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

h. a variant of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

i. an antigenic fragment of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, or SEQ ID NO: 97;

j. the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of

Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

k. a variant of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

l. an antigenic fragment of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19; or

m. a combination of one or more of the sequences in (a) – (l);

and wherein the variants or antigenic fragments of each said protein are immunoreactive with at least one antibody that binds to their corresponding peptide sequence.

41. An isolated or purified polypeptide, wherein said polypeptide comprises a sequence that is at least 95% identical to:

a. P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

b. a variant of P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

c. an antigenic fragment of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

d. SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

e. a variant of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

f. an antigenic fragment of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45;

g. SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

h. a variant of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

i. an antigenic fragment of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, or SEQ ID NO: 97;

j. the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

k. a variant of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about

position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

l. an antigenic fragment of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of

Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19; or

m. a combination of one or more of the sequences in (a) – (l);

and wherein the variants or antigenic fragments of each said protein are immunoreactive with at least one antibody that binds to their corresponding peptide sequence.

42. The isolated polypeptide of claims 40 or 41 wherein said polypeptide is the P44 Box 1 protein, a variant of the P44 Box 1 protein, or an antigenic fragment of the P44 Box 1 protein.

43. The isolated polypeptide of claims 40 or 41 wherein said polypeptide is the P44 Box 2 protein, a variant of the P44 Box 2 protein, or an antigenic fragment of the P44 Box 2 protein.

44. The isolated polypeptide of claims 40 or 41 wherein said polypeptide is the P44 Box 3 protein, a variant of the P44 Box 3 protein, or an antigenic fragment of the P44 Box 3 protein.

45. The isolated polypeptide of claims 40 or 41 wherein said polypeptide is the P44 Box 4 protein, a variant of the P44 Box 4 protein, or an antigenic fragment of the P44 Box 4 protein.

46. The isolated polypeptide of claims 40 or 41 wherein said polypeptide is the P44 Box 3 protein, a variant of the P44 Box 5 protein, or an antigenic fragment of the P44 Box 5 protein.

47. The isolated polypeptide of claims 40 or 41 wherein said polypeptide is the P44 Box 6 protein, a variant of the P44 Box 6 protein, or an antigenic fragment of the P44 Box 6 protein.

48. An isolated or purified polypeptide comprising a sequence chosen from the following: a Anaplasma platys OMP-1X protein, a variant of the Anaplasma platys OMP-1X protein, an antigenic fragment of the Anaplasma platys OMP-1X protein, wherein the Anaplasma platys OMP-1X protein comprises or consists of:

    a. OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

    b. a variant of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

    c. an antigenic fragment of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

    d. SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

    e. a variant of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

    f. an antigenic fragment of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or SEQ ID NO: 9;

    g. SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

    h. a variant of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

    i. an antigenic fragment of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, or SEQ ID NO: 8;

    j. the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the

amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

k. a variant of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

l. an antigenic fragment of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; or

m. a combination of one or more of the sequences in (a) – (l);

and wherein the variants or antigenic fragments of each said protein are immunoreactive with at least one antibody that binds to their corresponding peptide sequence.

49. An isolated or purified polypeptide, wherein said polypeptide comprises a sequence that is at least 95% identical to:

a. OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

b. a variant of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

c. an antigenic fragment of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

d. SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

e. a variant of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

f. an antigenic fragment of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or SEQ ID NO: 9;

g. SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

h. a variant of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

i. an antigenic fragment of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, or SEQ ID NO: 8;

j. the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

k. a variant of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

l. an antigenic fragment of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or fragment thereof; or

m. a combination of one or more of the sequences in (a) – (l);

and wherein the variants or antigenic fragments of each said protein are immunoreactive with at least one antibody that binds to their corresponding peptide sequence.

50. The isolated polypeptide of claims 48 or 49 wherein said polypeptide is the OMP-1X protein, a variant of the OMP-1X protein, or an antigenic fragment of the OMP-1X protein.

51. The isolated polypeptide of claims 48 or 49 wherein said polypeptide is the OMP-1X Box 1 protein, a variant of the OMP-1X Box 1 protein, or an antigenic fragment of the OMP-1X Box 1 protein.

52. The isolated polypeptide of claims 48 or 49 wherein said polypeptide is the OMP-1X Box 2 protein, a variant of the OMP-1X Box 2 protein, or an antigenic fragment of the OMP-1X Box 2 protein.

53. An isolated or purified antibody that selectively to a peptide chosen from:

a. *Anaplasma platys* P44 protein, *Anaplasma platys* OMP-1X protein, P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof; or

b. one or more of the peptide sequences of claims 40-52 or 54-55.

54. A peptide comprising one or more amino-acid sequences selected from the group consisting of:

a. P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

b. a variant of P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

c. an antigenic fragment of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

d. SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

e. a variant of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

f. an antigenic fragment of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45;

g. SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

h. a variant of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

i. an antigenic fragment of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, or SEQ ID NO: 97;

j. the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of

Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

k. a variant of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

l. an antigenic fragment of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about

position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof; or

m. a combination of one or more of the sequences in (a) – (l);

or any antigenically related variants of said sequences which have an identity of 95% and are capable of immunologically mimicking the corresponding antigenic determinant site of the P44 protein of *Anaplasma platys*.

55. A peptide comprising one or more amino-acid sequences selected from the group consisting of:

a. OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

b. a variant of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

c. an antigenic fragment of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

d. SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

e. a variant of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

f. an antigenic fragment of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or SEQ ID NO: 9;

g. SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

h. a variant of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

i. an antigenic fragment of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, or SEQ ID NO: 8;

j. the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

k. a variant of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of

Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

l. an antigenic fragment of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; or

m. a combination of one or more of the sequences in (a) – (l);

or any antigenetically related variants of said sequences which have an identity of 95% and are capable of immunologically mimicking the corresponding antigenetic determinant site of the OMP-1X protein *of Anaplasma platys*.

56. A chimeric peptide comprising one or more peptides of any of claims 40-52 or 54-55 covalently linked to a carrier polypeptide which comprises at least one T-cell epitope.

57. The chimeric peptide of any of claims 40-52 or 54-56 which also comprises a purification tag peptide sequence.

58. The chimeric polypeptide of claim 57 wherein the purification tag peptide sequence is a Histidine-tag sequence.

59. The chimeric polypeptide of any of claims 40-52 or 54-56 which also comprises a Histidine purification tag sequence.

60. A purified antibody which is immunospecific to a peptide provided in any of claims 56-59.

61. A purified antibody which is immunospecific to a chimeric polypeptide provided in claims 56-59.

62. A vaccine composition comprising an immunogenic amount of at least one peptide or polypeptide of any of claims 40-52 or 54-59 in a pharmaceutically acceptable excipient, and an optional adjuvant.

63. A method of inducing an immune response in a mammal susceptible to *Anaplasma platys* infection comprising the administration to the mammal of an effective amount of the vaccine according to claim 62.

64. A method of preventing *Anaplasma platys* infection comprising the administration to a mammal an effective amount of a vaccine according to claim 62.

65. A method of detecting the presence of *Anaplasma platys* in a sample by contacting said sample with the antibody of claims 53, 60 or 61 in the presence of an indicator.

66. A method of detecting the presence of *Anaplasma platys* in a sample by contacting said sample with a DNA probe or primer constructed to correspond to the P44 protein of *Anaplasma platys*, characterized in that the probe or primer comprises one or more of the nucleotides of any of claims 18-20, 24-27 or 33-34.

67. A method of detecting the presence of *Anaplasma platys* in a sample by contacting said sample with a DNA probe or primer constructed to correspond to the OMP-1X protein, OMP-1X Box 1, or OMP-1X Box 2 of *Anaplasma platys*, characterised in that the probe or primer comprises one or more of the nucleotides of any of claims 21-23, 28-31 or 33-34.

68. A method for diagnosing an infection with *Anaplasma platys* in a subject comprising the steps of: (a) providing a serum sample from the subject; (b) providing an isolated or purified P44 protein of *Anaplasma platys*; (c) contacting the serum sample with the P44 protein; and (d) assaying for the formation of a complex between antibodies in the serum sample and the P44 protein, wherein formation of said complex is indicative of infection with *Anaplasma platys*.

69. The method of claim 68 wherein the *Anaplasma platys* P44 protein comprises P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof.

70. The method of claim 68 wherein the *Anaplasma platys* P44 protein comprises a variant of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof.

71. The method of claim 68 wherein the *Anaplasma platys* P44 protein comprises an antigenic fragment of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof.

72. The method of claim 68 wherein the *Anaplasma platys* P44 protein comprises one or more of the peptide sequences of claims 40 – 47 or 54.

73. A method for diagnosing an infection with *Anaplasma platys* in a subject comprising the steps of: (a) providing a serum sample from the subject; (b) providing an isolated or purified OMP-1X protein of *Anaplasma platys*; (c) contacting the serum sample with the OMP-1X protein; and (d) assaying for the formation of a complex between antibodies in the serum sample and the OMP-1X protein, wherein formation of said complex is indicative of infection with *Anaplasma platys*.

74. The method of claim 73 wherein the *Anaplasma platys* P44 protein comprises OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof.

75. The method of claim 73 wherein the *Anaplasma platys* P44 protein comprises a variant of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof.

76. The method of claim 73 wherein the *Anaplasma platys* P44 protein comprises an antigenic fragment of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof.

77. The method of claim 73 wherein the *Anaplasma platys* OMP-1X protein comprises one or more of the peptide sequences of claims 48-52 or 55.

78. A kit for diagnosing *Anaplasma platys* in a subject, said kit comprising the the *Anaplasma platys* P44 protein, an antigenic fragment of the *Anaplasma platys* P44 protein, or both.

79. The kit of claim wherein said *Anaplasma platys* P44 protein or antigenic fragment of the *Anaplasma platys* P44 protein comprises one or more of the peptide sequences of claims 40-47 or 54.

80. A kit for diagnosing *Anaplasma platys* in a subject, said kit comprising the the *Anaplasma platys* OMP-1X protein, an antigenic fragment of the *Anaplasma platys* OMP-1X protein, or both.

81. The kit of claim wherein said *Anaplasma platys* OMP-1X protein or antigenic fragment of the *Anaplasma platys* OMP-1X protein comprises one or more of the peptide sequences of claims 48-52 or 55.

82. The kit of any of claims 78-81, further comprising a biomolecule for detecting interaction between the reagent and antibodies in a bodily sample of the animal.

83. A reagent kit for diagnosing infection with *Anaplasma platys* in a subject comprising a DNA probe or primer constructed to correspond to the P44 protein, P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, or P44 Box 6 of *Anaplasma platys*, characterized in that the probe or primer comprises one or more of the nucleotides of any of claims 18-20, 24-27, or 33-35.

84. A reagent kit for diagnosing infection with *Anaplasma platys* in a subject comprising a DNA probe or primer constructed to correspond to the OMP-1X protein, OMP-1X Box 1, or OMP-1X Box 2 of *Anaplasma platys*, characterized in that the probe or primer comprises one or more of the nucleotides of any of claims 21-23, 28 -31, or 33-35.

85. A reagent kit for diagnosing infection with *Anaplasma platys* in a subject comprising one or more of the antibodies of claims 53, 60, or 61.

86. A method of detecting antibodies that specifically bind an *Anaplasma platys* polypeptide, comprising:

    a. contacting a purified polypeptide comprising the amino acid sequence of one or more of the following:

i. P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

ii. a variant of P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

iii. an antigenic fragment of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

iv. SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

v. a variant of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

vi. an antigenic fragment of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45;

vii. SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

viii. a variant of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

ix. an antigenic fragment of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, or SEQ ID NO: 97;

x. the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

xi. a variant of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid

sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

xii. an antigenic fragment of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure

23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

xiii. a combination of one or more of the sequences in (i) – (xii);

xiv. OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

xv. a variant of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

xvi. an antigenic fragment of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

xvii. SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

xviii. a variant of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

xix. an antigenic fragment of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or SEQ ID NO: 9;

xx. SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

xxi. a variant of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

xxii. an antigenic fragment of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, or SEQ ID NO: 8;

xxiii. the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

xxiv. a variant of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

xxv. an antigenic fragment of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; or

xxvi. a combination of one or more of the sequences in (xiv) – (xxv);

with a test sample, under conditions that allow polypeptide/antibody complexes to form;

b. detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that antibodies specific for an *Anaplasma platys* polypeptide is present in the test sample.

87. The method of claim 86, further comprising contacting the complexes of step (a) with an indicator reagent prior to the performance of step (b).

88. The method of claim 86, wherein the amount of antibody in the test sample is determined.

89. The method of claim 86, wherein the purified polypeptide is attached to a substrate.

90. The method of claim 86, wherein the purified polypeptide is a fusion protein, wherein the purified polypeptide is fused to an indicator reagent, an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, a transmembrane domain, a protein purification ligand, a heterologous protein, or a combination thereof.

91. The method of claim 86, wherein the purified polypeptide is in multimeric form.

92. The method of claim 86, wherein the method comprises a microtiter plate assay, reversible flow chromatographic binding assay, an enzyme linked immunosorbent

assay, a radioimmunoassay, a hemagglutination assay, a western blot assay, a fluorescence polarization immunoassay, or an indirect immunofluorescence assay.

93. The method of claim 86, wherein the test sample is a biological sample from a subject, and wherein the detection of polypeptide/antibody complexes is an indication that the subject has an Anaplasma platys infection or has been exposed to *Anaplasma platys*.

94. A method of detecting an *Anaplasma platys* infection or exposure to *Anaplasma platys* in a subject comprising: (a) obtaining a biological sample from the subject; (b) contacting a purified polypeptide comprising the amino acid sequence of one or more of the following:

i. P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

ii. a variant of P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

iii. an antigenic fragment of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

iv. SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

v. a variant of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

vi. an antigenic fragment of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID

NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45;

vii. SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

viii. a variant of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

ix. an antigenic fragment of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, or SEQ ID NO: 97;

x. the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid

from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

xi. a variant of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising

the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

xii. an antigenic fragment of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

xiii. a combination of one or more of the sequences in (i) – (xii);

xiv. OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

xv. a variant of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

xvi. an antigenic fragment of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

xvii. SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

xviii. a variant of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

xix. an antigenic fragment of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or SEQ ID NO: 9;

xx. SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

xxi. a variant of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

xxii. an antigenic fragment of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, or SEQ ID NO: 8;

xxiii. the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

xxiv. a variant of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about

position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

xxv. an antigenic fragment of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; or

xxvi. a combination of one or more of the sequences in (xiv) – (xxv);

with the biological sample under conditions that allow polypeptide/antibody complexes to form; and (c) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that the subject has an *Anaplasma platys* infection or exposure to *Anaplasma platys*.

95. The method of claim 94, further comprising contacting the polypeptide/antibody complexes of step (b) with an indicator reagent that generates a measurable signal prior to the performance of step (c).

96. The method of claim 94, wherein the purified polypeptide is a fusion protein wherein the purified polypeptide is fused to an indicator reagent, an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, a transmembrane domain, a protein purification ligand, a heterologous protein or a combination thereof.

97. The method of claim 94, wherein the polypeptide/antibody complexes are detected at about 10 days after exposure or infection of subject by Anaplasma platys.

98. A method of detecting *Anaplasma platys* polypeptides in a test sample comprising: (a) contacting one or more antibodies that specifically bind to a Anaplasma platys polypeptide with the test sample under conditions that allow polypeptide/antibody complexes to form; wherein the Anaplasma platys polypeptide comprises the amino acid sequence of one or more of the following:

    i. P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

    ii. a variant of P44 Box 1, P44, Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

    iii. an antigenic fragment of P44 Box 1, P44 Box 2, P44 Box 3, P44 Box 4, P44 Box 5, P44 Box 6, or a fragment thereof;

    iv. SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

    v. a variant of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, or a fragment thereof;

    vi. an antigenic fragment of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, or SEQ ID NO: 45;

vii. SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

viii. a variant of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97, or a fragment thereof;

ix. an antigenic fragment of SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, or SEQ ID NO: 97;

x. the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to

about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

xi. a variant of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

xii. an antigenic fragment of the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, or a fragment thereof;

xiii. a combination of one or more of the sequences in (i) – (xii);

xiv. OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

xv. a variant of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

xvi. an antigenic fragment of OMP-1X Box 1, OMP-1X Box 2, or a fragment thereof;

xvii. SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

xviii. a variant of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, or a fragment thereof;

xix. an antigenic fragment of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or SEQ ID NO: 9;

xx. SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

xxi. a variant of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, or a fragment thereof;

xxii. an antigenic fragment of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, or SEQ ID NO: 8;

xxiii. the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

xxiv. a variant of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about

position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof;

xxv. an antigenic fragment of the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, or a fragment thereof; or

xxvi. a combination of one or more of the sequences in (xiv) – (xxv);

(b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that an Anaplasma platys polypeptide is present in the test sample.

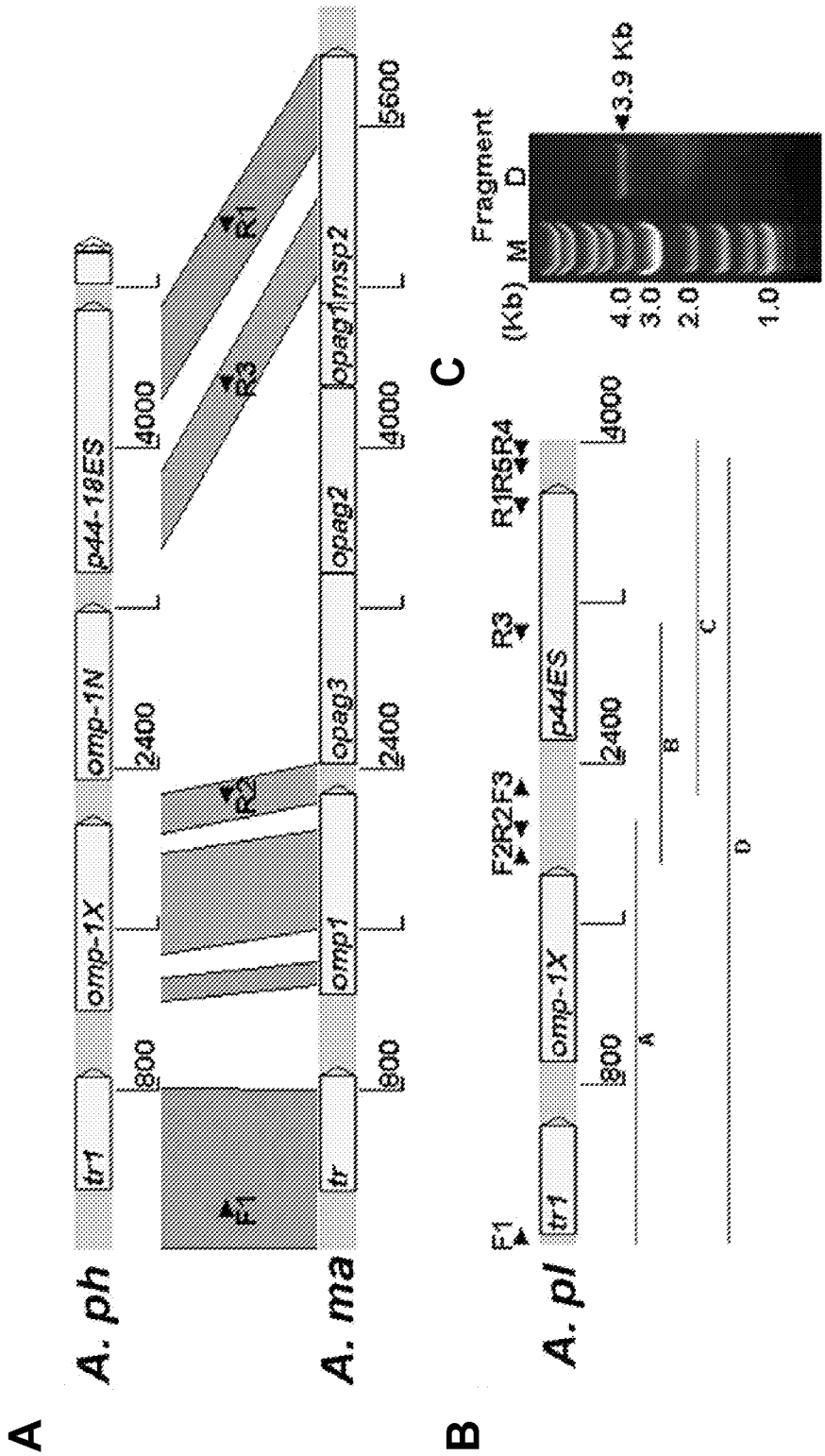
99. The method of claim 98, wherein the one or more antibodies are monoclonal antibodies, polyclonal antibodies, Fab fragments, Fab' fragments, Fab'-SH fragments, $F(ab')_2$ fragments, Fv fragments, or single chain antibodies.

100. The method of claim 98, further comprising contacting the complexes of step (a) with an indicator reagent prior to the performance of step (b).

101. The method of claim 98, wherein the amount of *Anaplasma platys* polypeptides in the test sample is determined.

102. The method of claim 98, wherein the one or more antibodies are attached to a substrate.

103. The method of claim 98, wherein the method comprises a microtiter plate assay, reversible flow chromatographic binding assay, an enzyme linked immunosorbent assay, a radioimmunoassay, a hemagglutination assay, a western blot assay, a fluorescence polarization immunoassay, or an indirect immunofluorescence assay.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R3
R2
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omp1
opag3
opag2
opag1msp2
800
2400
4000
5600
B
A. pl
tr1
omp-1X
p44ES
F1
F2R2F3
R3
R1R5R4
800
2400
4000
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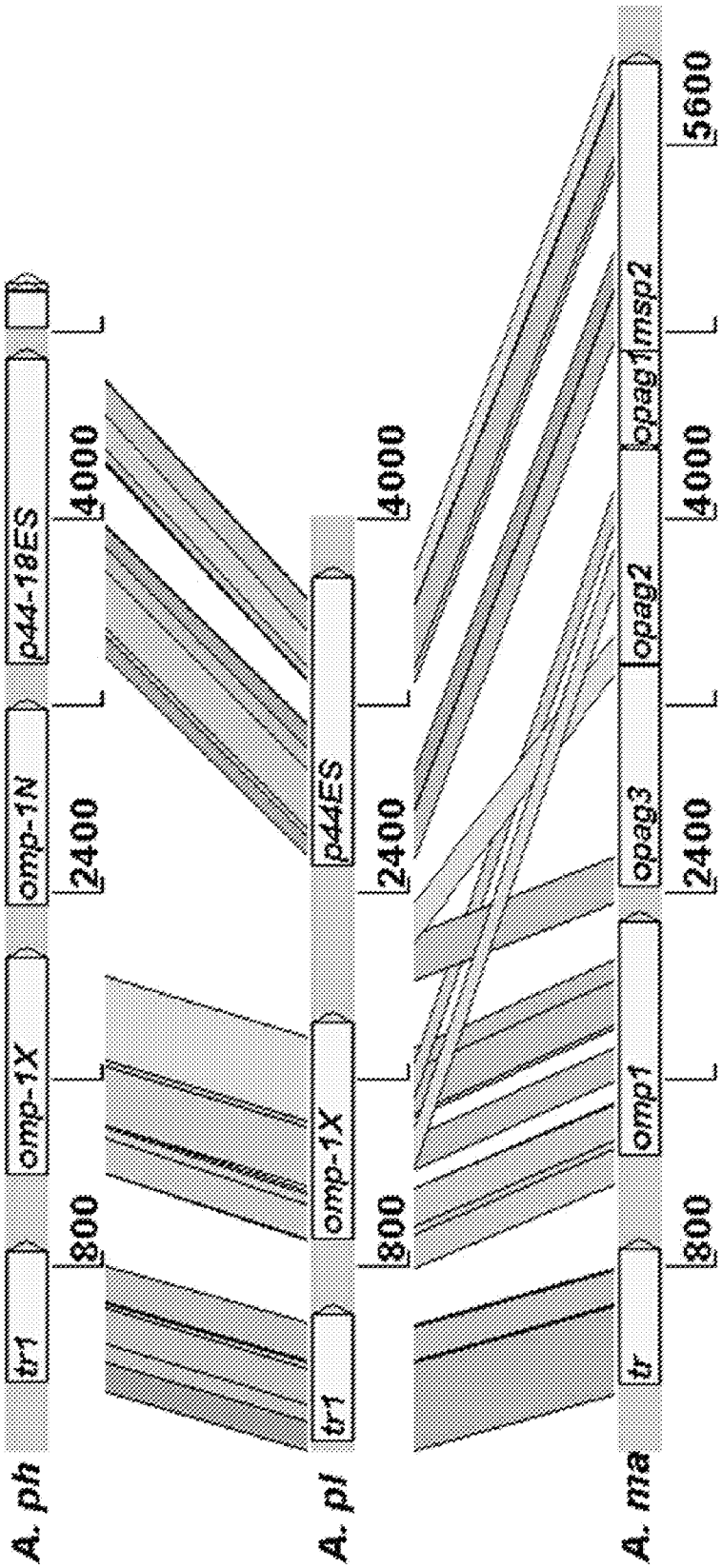
3.0
2.0
1.0
3.9 Kb

Figure 1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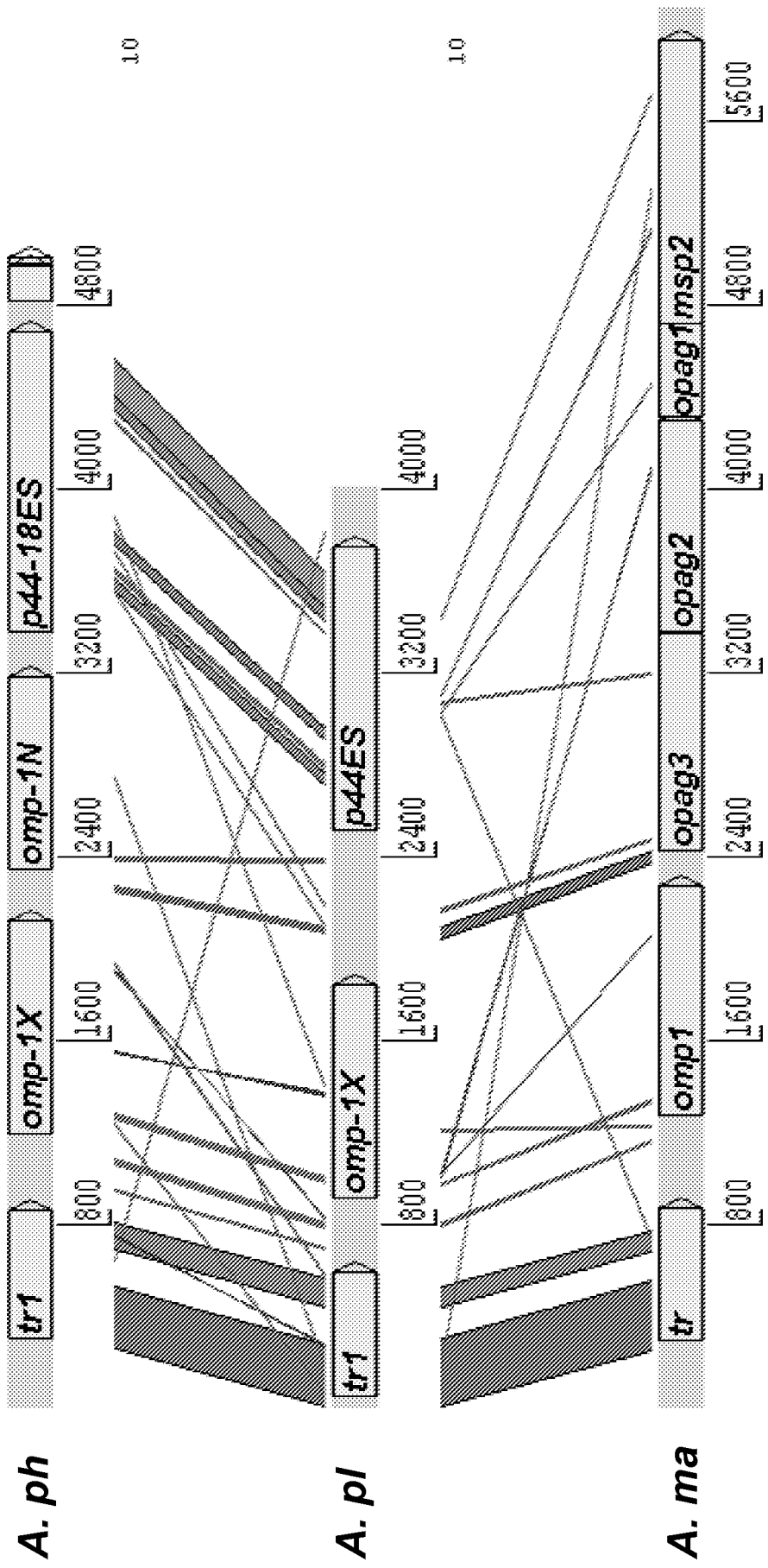
4000
opag2
5600
opag1msp2

Figure 2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4800
1
0
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omp-1X
p44ES
800
1600
2400
3200
4000
1
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2400
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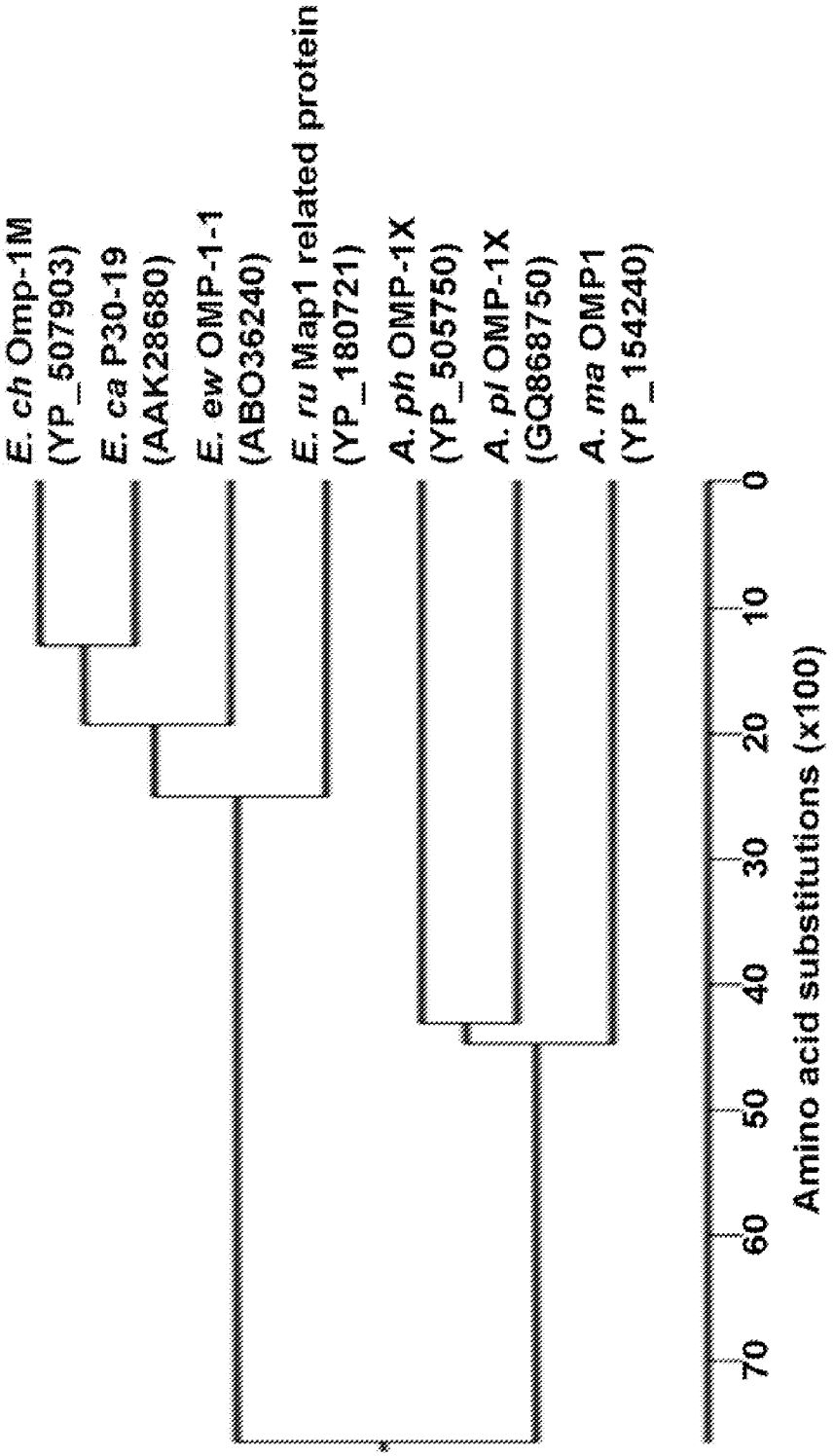
4000
4800
5600

Figure 3


E. ch Omp-1M
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E. ca P30-19
(AAK28680)
E. ew OMP-1-1
(ABO36240)
E. ru Map1 related protein
(YP_180721)
A. ph OMP-1X
(YP_505750)
A. pl OMP-1X
(GQ868750)
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(YP_154240)
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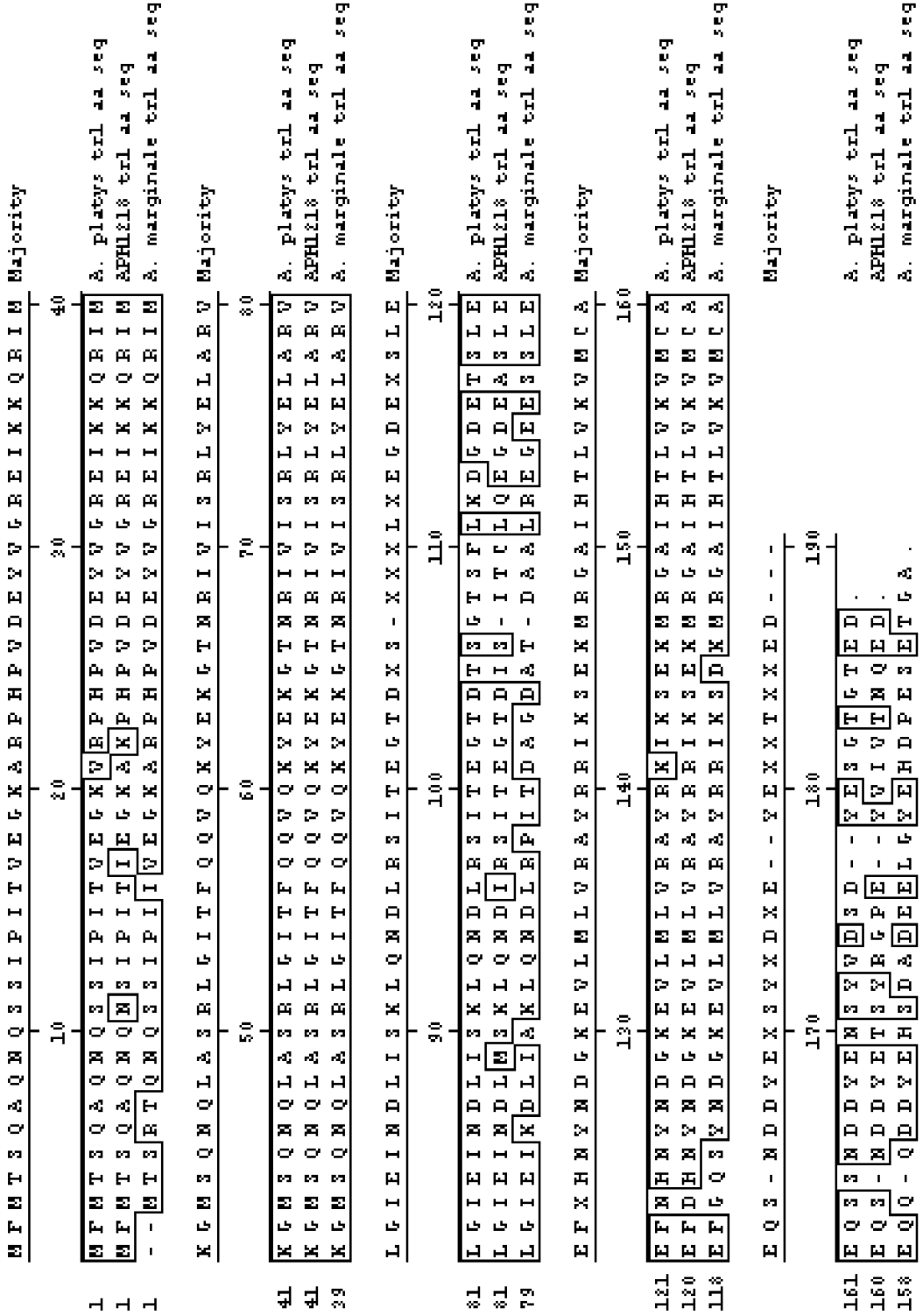
10
20
30
40
50
60
70
Amino acid substitutions (x100)

Figure 4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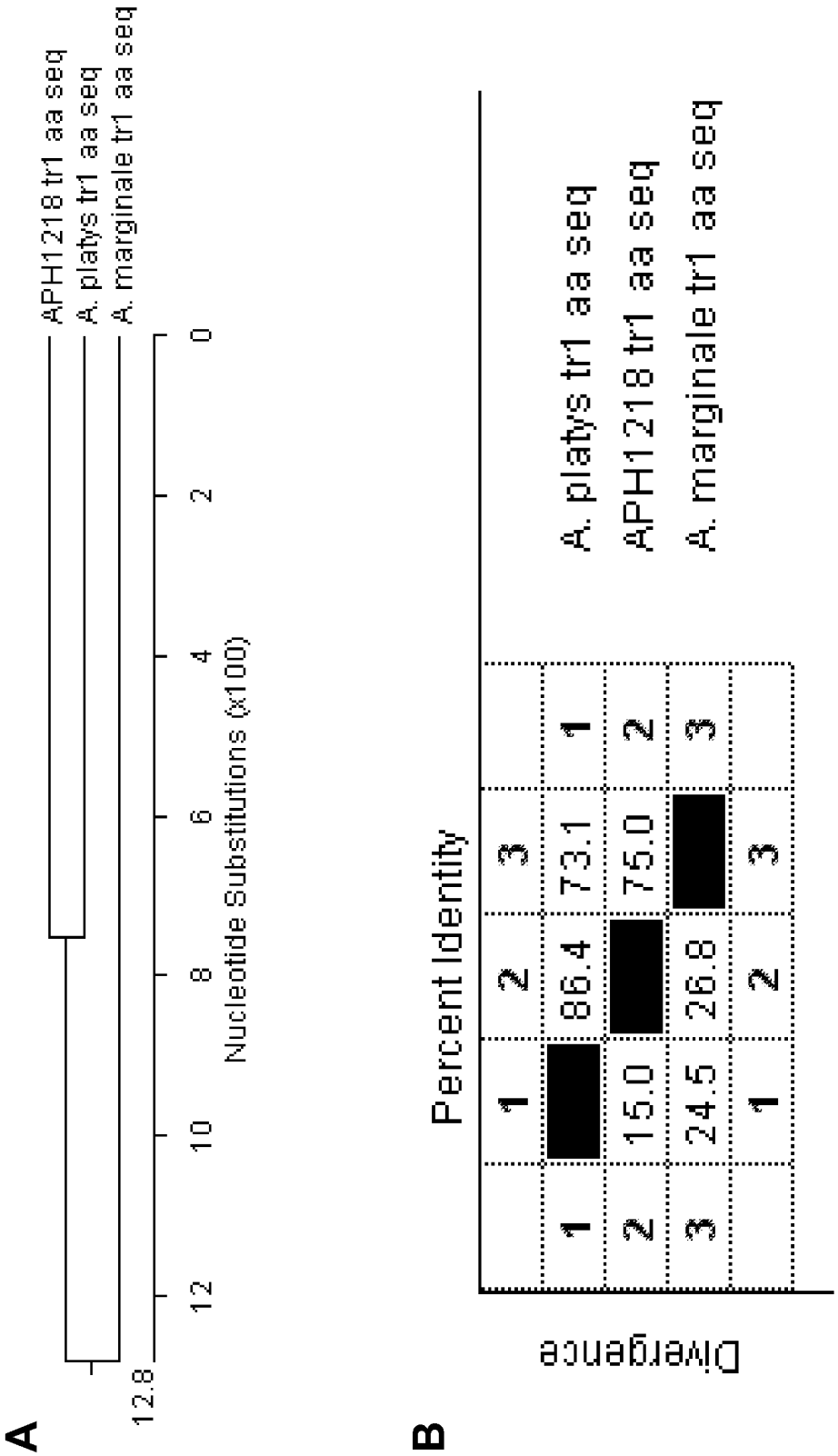
APHid18 trl aa seq
a. marginale trl aa seq
Decoration '#1': Box residues that match the Consensus exactly.

Figure 5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APH1218 tr1 aa seq
A. platys tr1 aa seq
A. marginale tr1 aa seq
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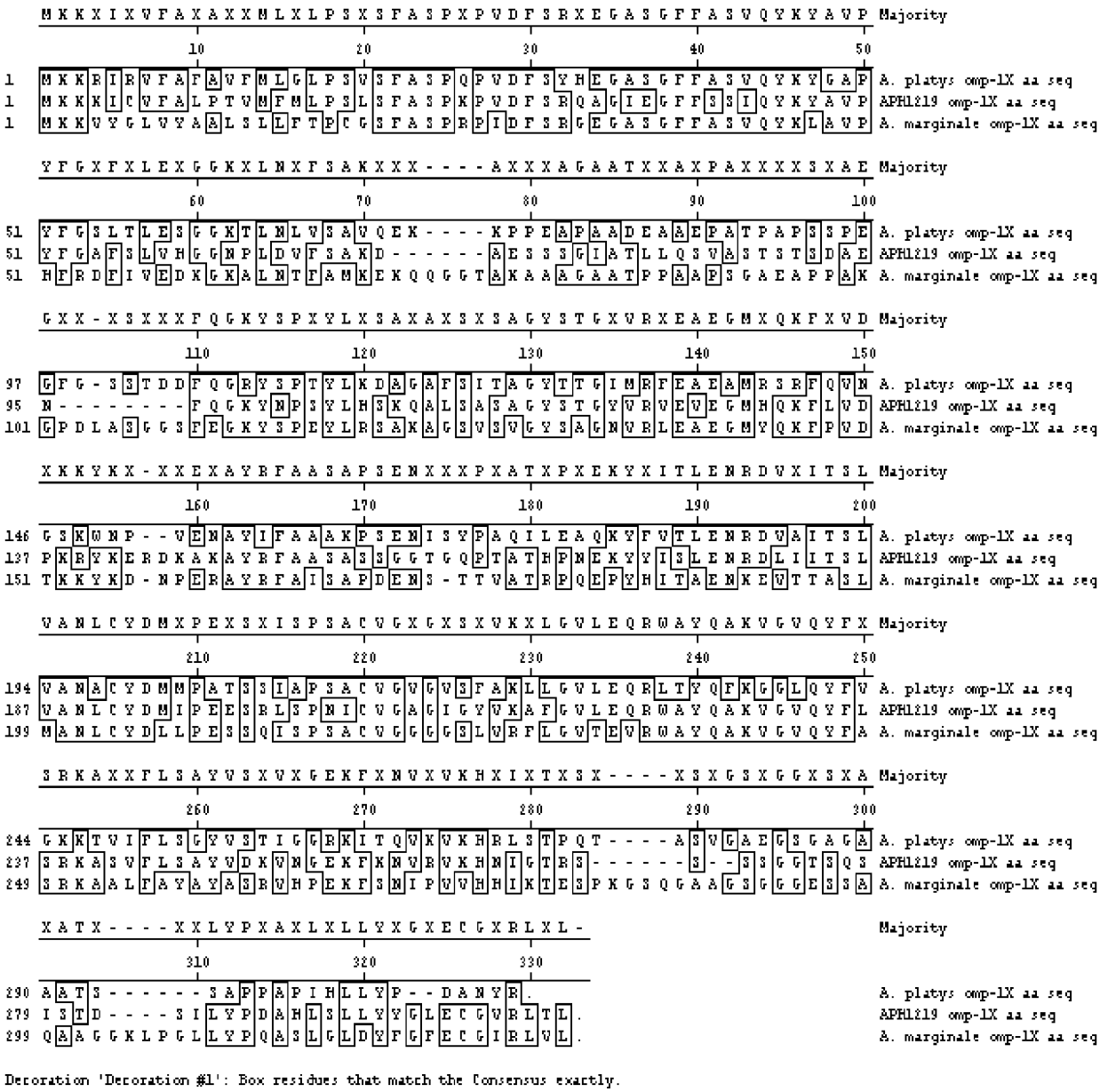
12
10
8
6
4
2
0
Nucleotide Substitutions (x100)
B
Percent Identity
Divergence
1
2
3
86.4
73.1
75.0
15.0
24.5
26.8
A. platys tr1 aa seq
APH1218 tr1 aa seq
A. marginale tr1 aa seq

**Figure 6**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APH1219 omp-1X aa seq
A. marginale omp-1X aa seq
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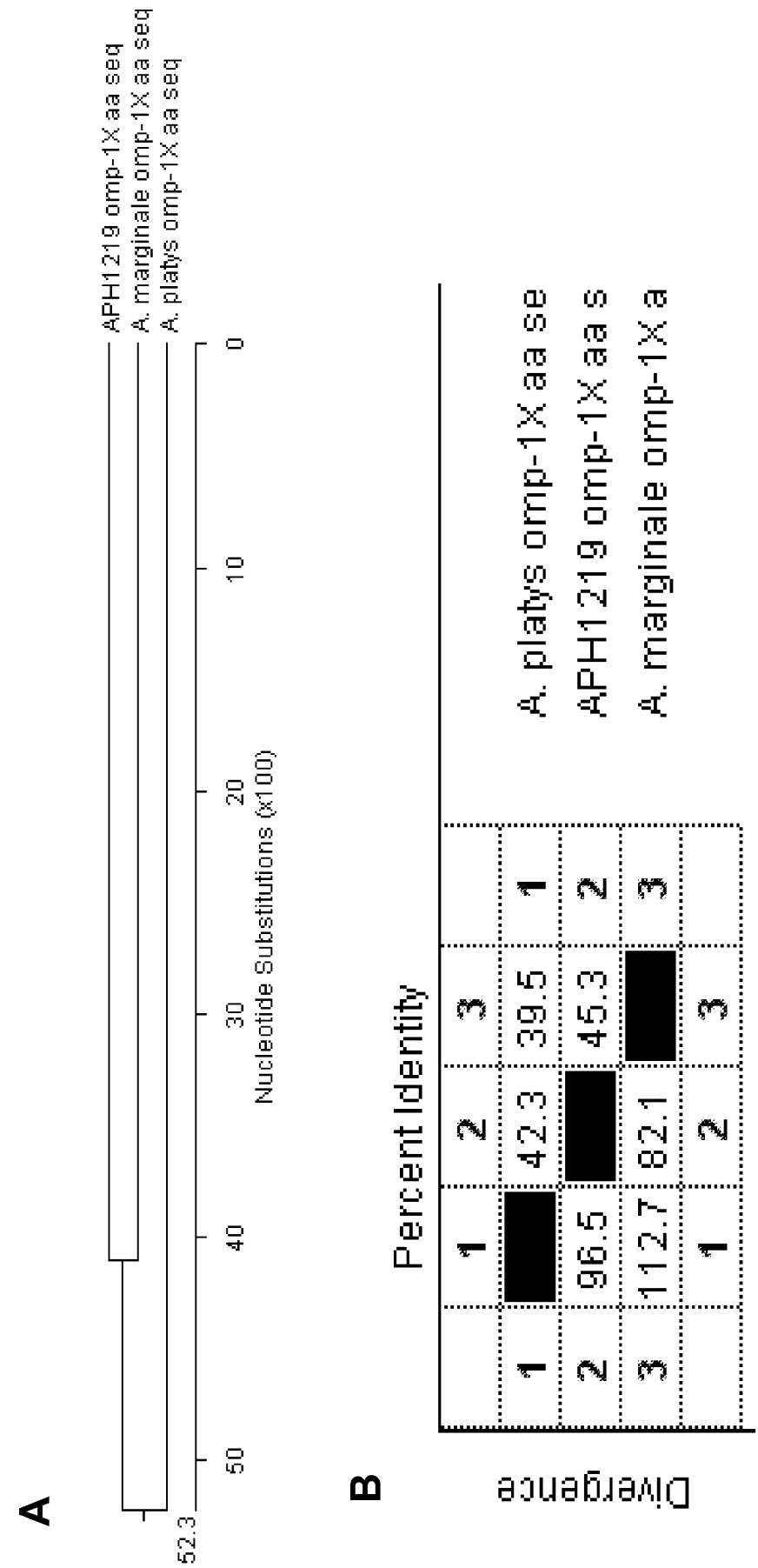
160 170 180 190 200
210 220 230 240 250
260 270 280 290 300
310 320 330
Decoration 'Decoration #1': Box residues that match the Consensus exactly.

Figure 7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APH1219 omp-1X aa seq
A. marginale omp-1X aa seq
A. platys omp-1X aa seq
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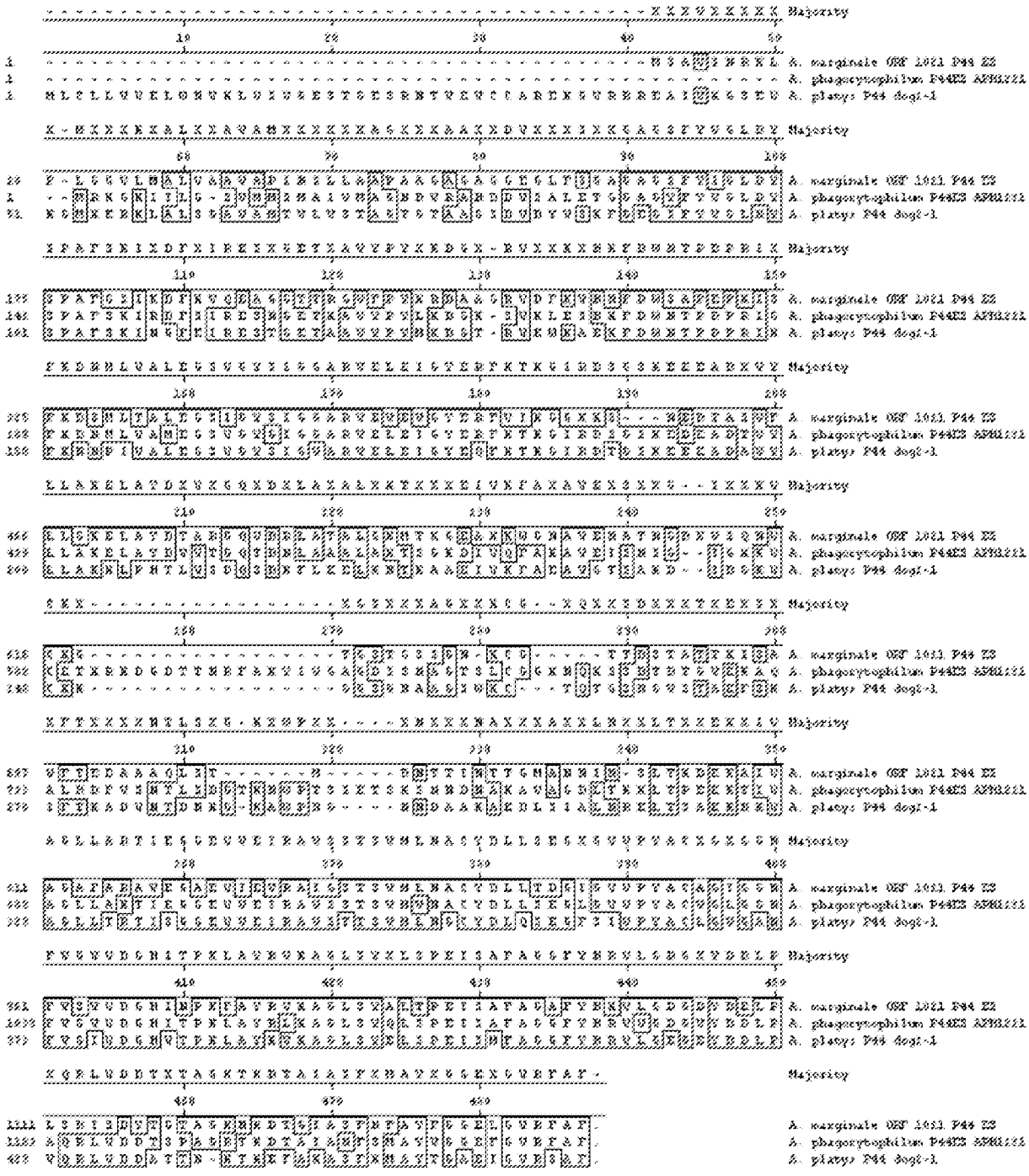
50
40
30
20
10
0
Nucleotide Substitutions (x100)
B
Percent Identity
Divergence
1
2
3
1
96.5
112.7
42.3
82.1
39.5
45.3
A. platys omp-1X aa se
APH1219 omp-1X aa s
A. marginale omp-1X a

Figure 8



# Figure 9

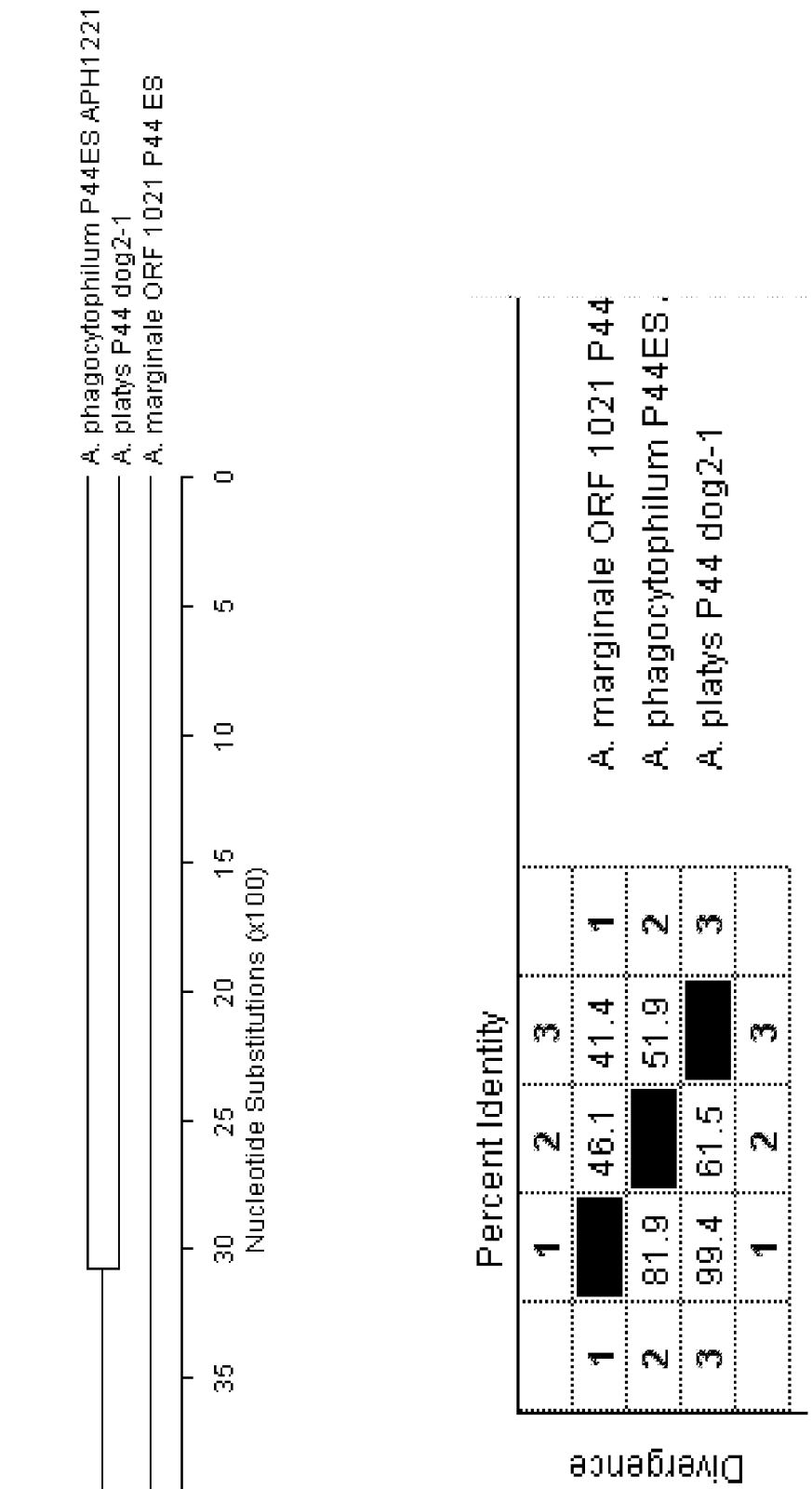

A
A. phagocytophilum P44ES APH1221
A. platys P44 dog2-1
A. marginale ORF 1021 P44 ES
45.3
45
40
35
30
25
20
15
10
5
0
Nucleotide Substitutions (x100)
B
Percent Identity
Divergence
1
2
3
1
46.1
41.4
2
81.9
51.9
3
99.4
61.5
1
2
3
A. marginale ORF 1021 P44
A. phagocytophilum P44ES
A. platys P44 dog2-1

**Figure 10**

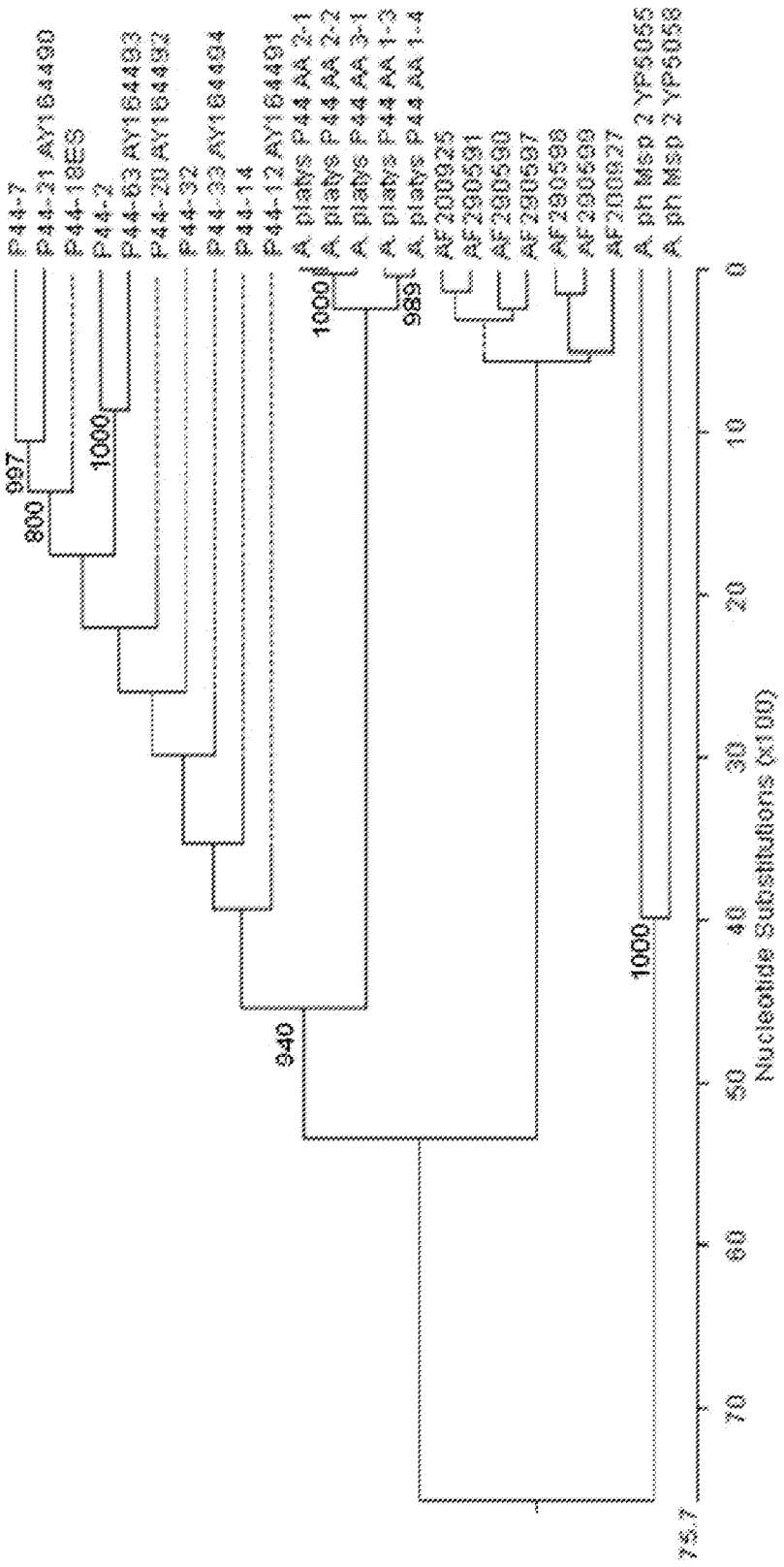

P44-7
P44-31 AY164490
P44-18ES
P44-2
P44-63 AY164493
P44-20 AY164492
P44-32
P44-33 AY164494
P44-14
P44-12 AY164491
A. platys P44 AA 2-1
A. platys P44 AA 2-2
A. platys P44 AA 3-1
A. platys P44 AA 1-3
A. platys P44 AA 1-4
AF200525
AF290591
AF290590
AF290587
AF290588
AF290589
AF200527
A. ph Msp 2 YP5055
A. ph Msp 2 YP5058
997
800
1000
1000
989
940
1000
75.7
0
10
20
30
40
50
60
70
Nucleotide Substitutions (x100)

Figure 11

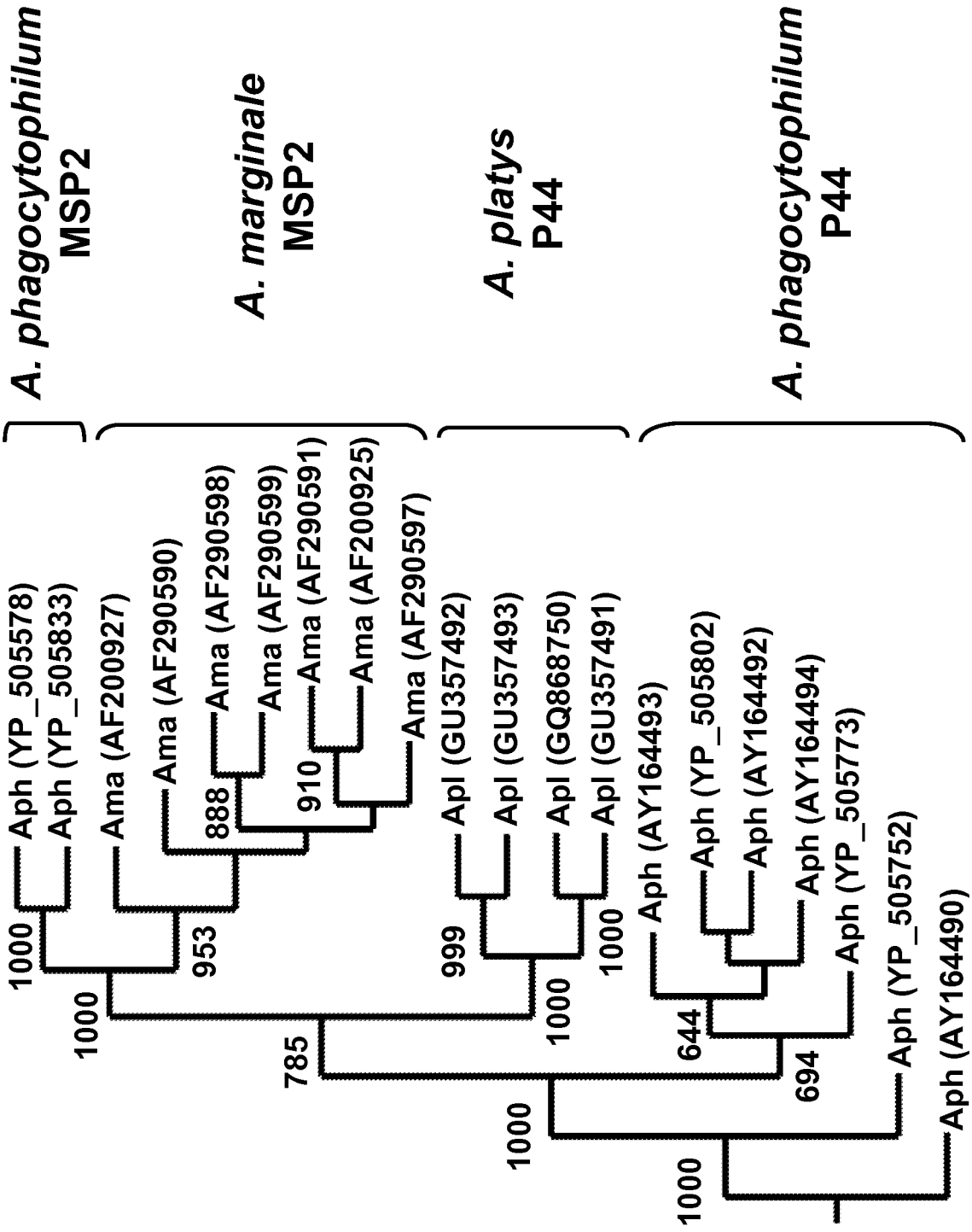

A. phagocytophilum MSP2
A. marginale MSP2
A. platys P44
A. phagocytophilum P44
Aph (YP_505578)
Aph (YP_505833)
Ama (AF200927)
Ama (AF290590)
Ama (AF290598)
Ama (AF290599)
Ama (AF290591)
Ama (AF200925)
Ama (AF290597)
Apl (GU357492)
Apl (GU357493)
Apl (GQ868750)
Apl (GU357491)
Aph (AY164493)
Aph (YP_505802)
Aph (AY164492)
Aph (AY164494)
Aph (YP_505773)
Aph (YP_505752)
Aph (AY164490)
1000
1000
953
888
910
1000
999
1000
1000
785
644
694
1000

Figure 12

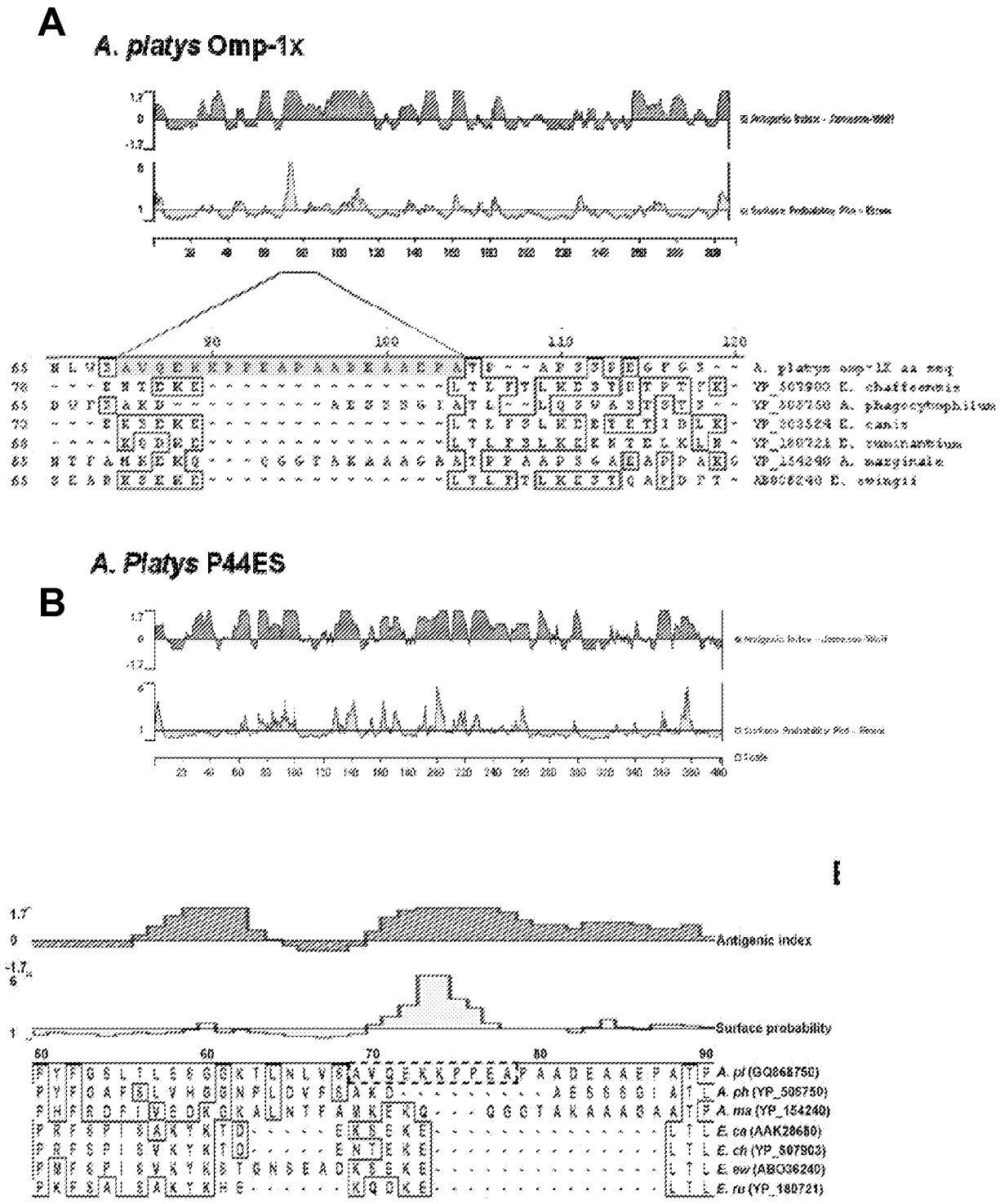

A
A. platys Omp-1x
B
A. Platys P44ES
Antigenic index
Surface probability

Figure 13

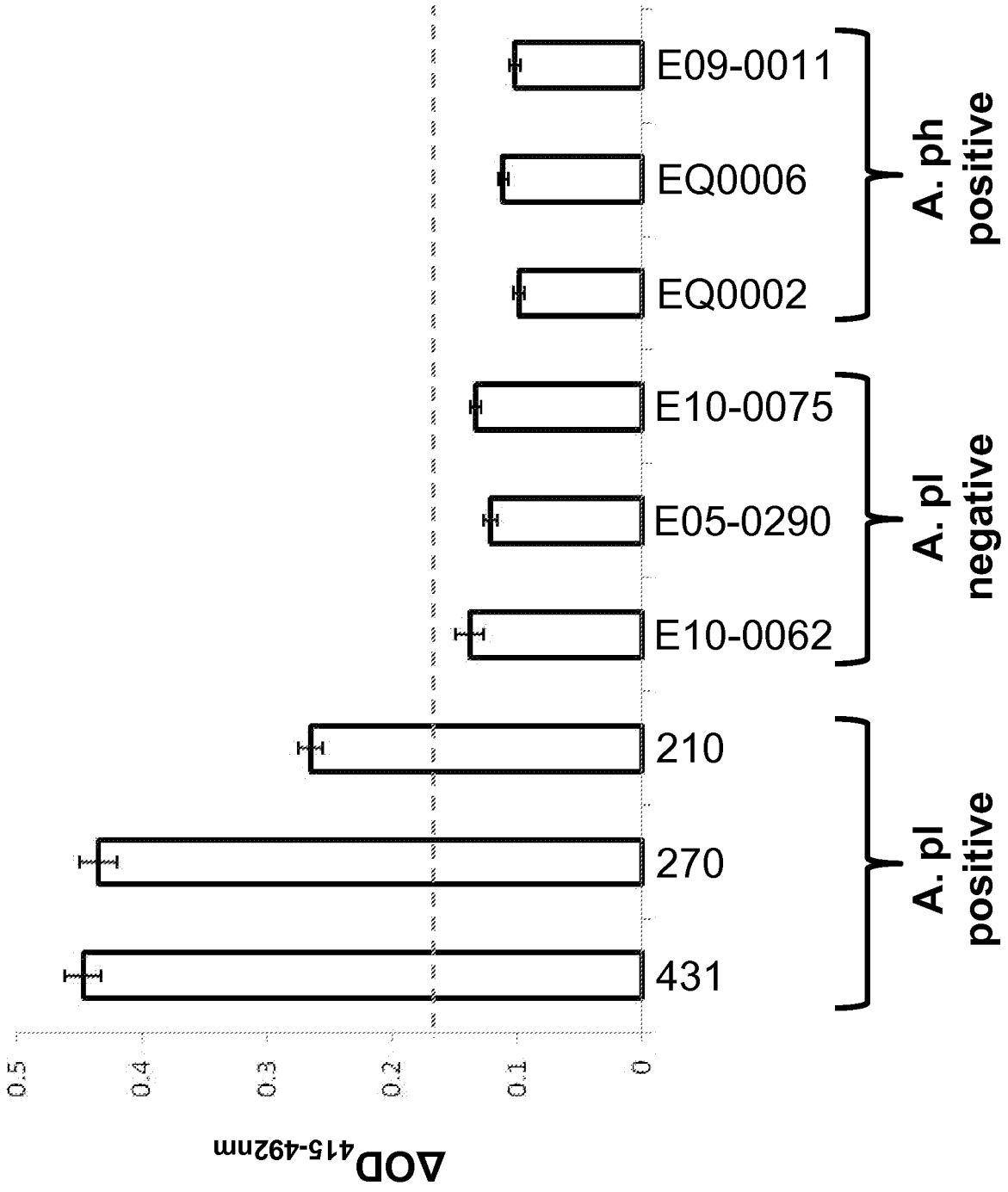

ΔOD415-492nm
0.5
0.4
0.3
0.2
0.1
0
431
270
210
E10-0062
E05-0290
E10-0075
EQ0002
EQ0006
E09-0011
A. pl positive
A. pl negative
A. ph positive

Figure 14

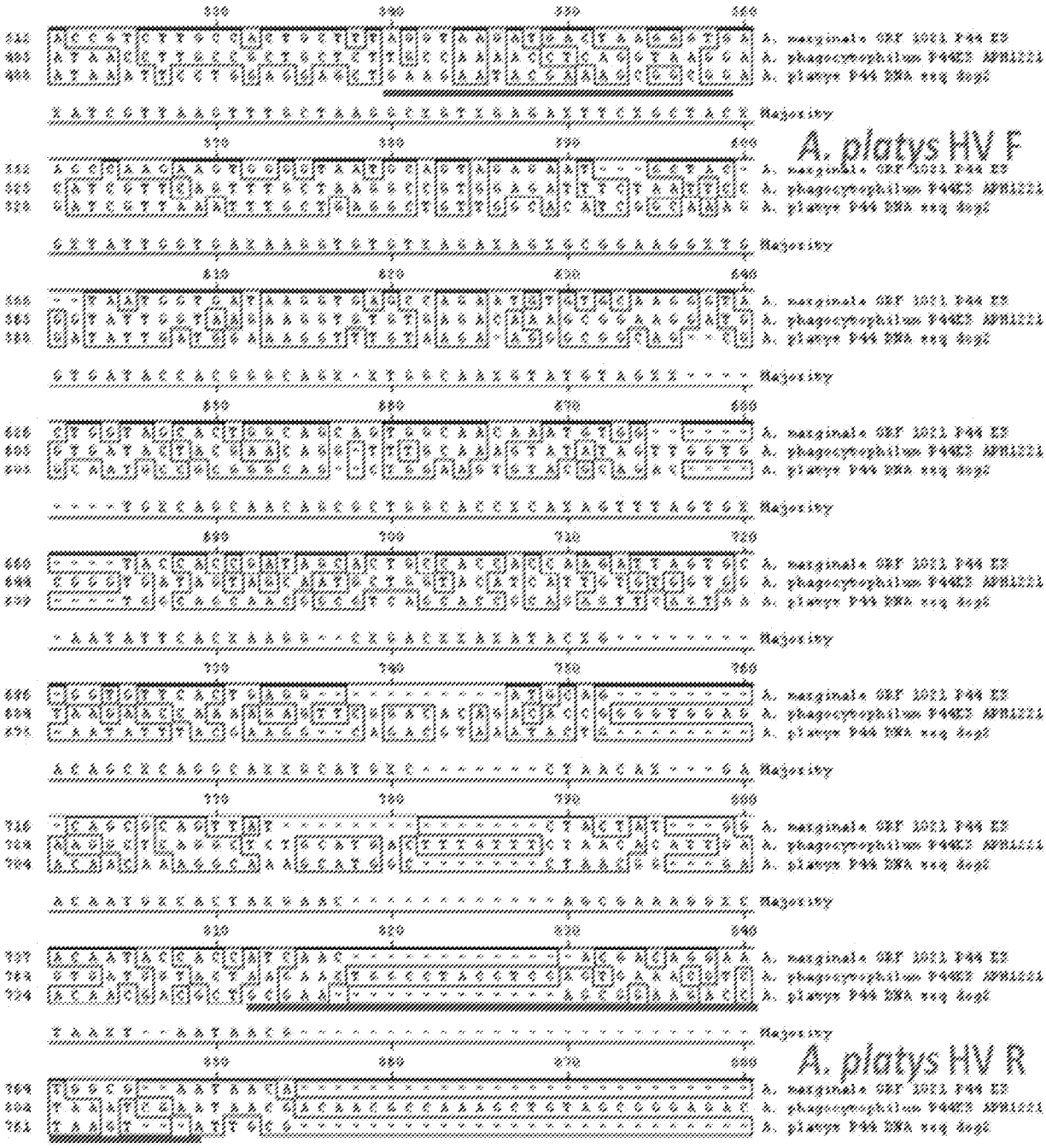



Figure 15

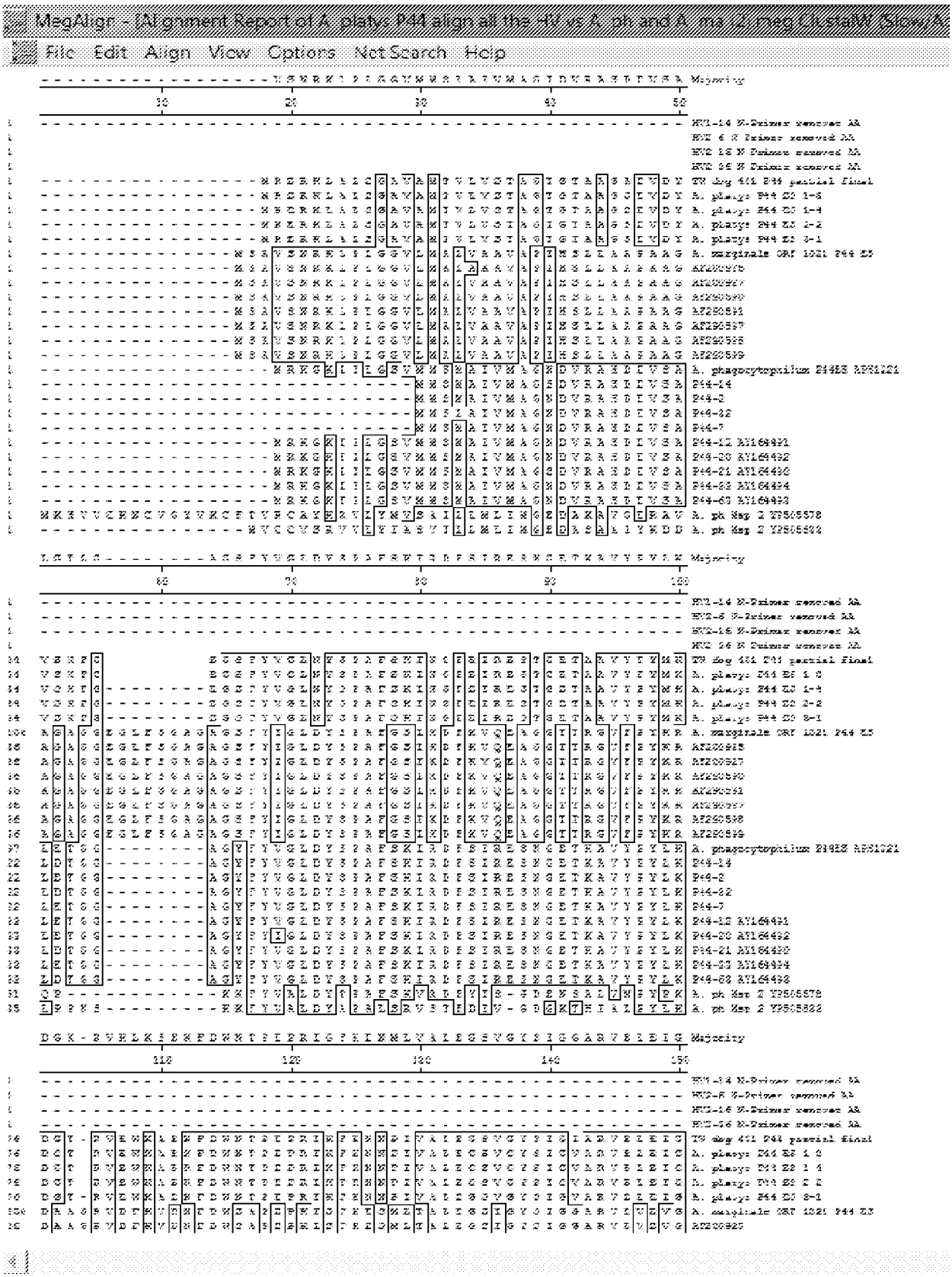

MegAlign - [Alignment Report of A. platys P44 align all the 4V vs A. ph and A. ma (2) meg Clustal W (Slow) A
File Edit Align View Options Nuc Search Help

Figure 16

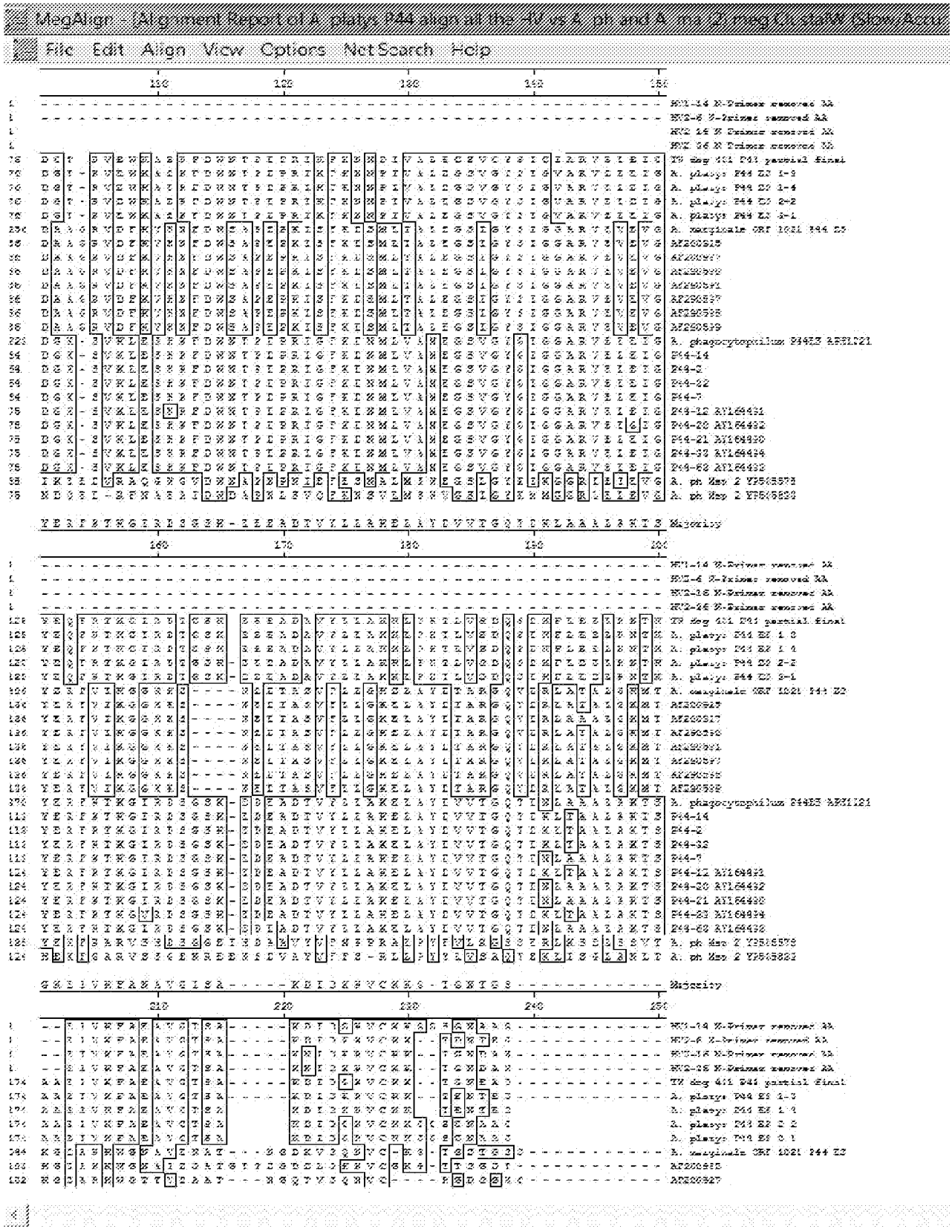



Figure 16 (continued)

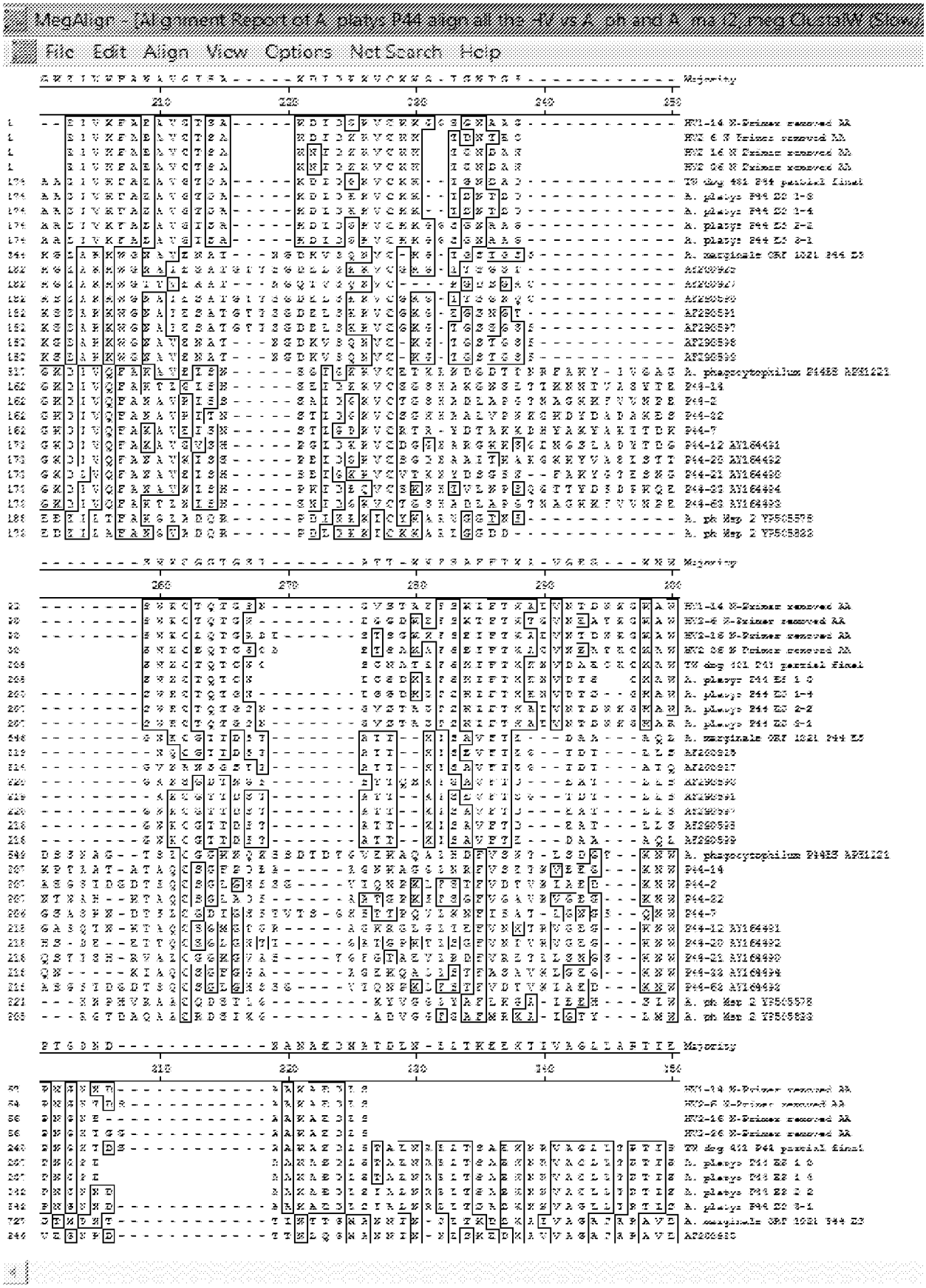



Figure 16 (continued)

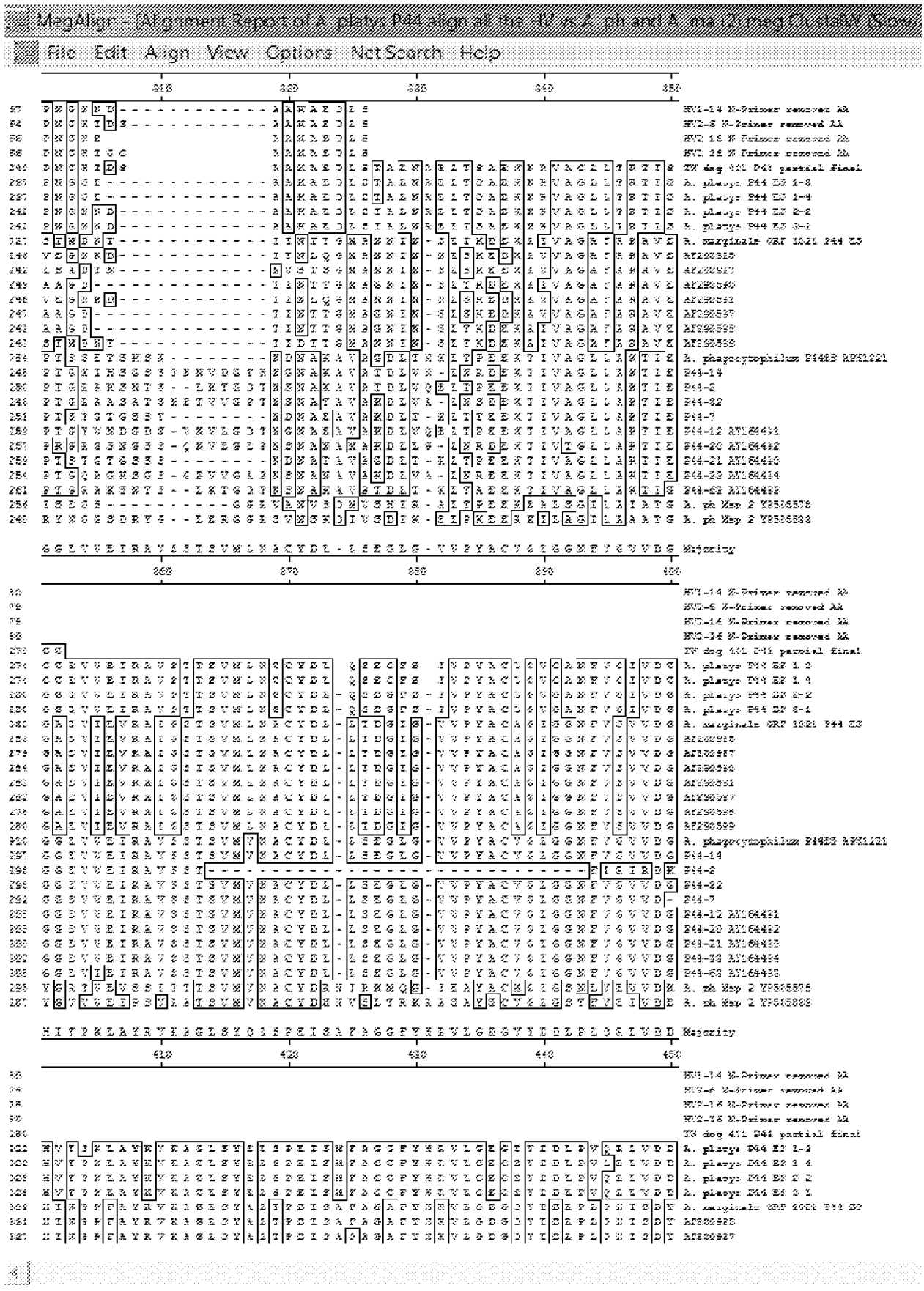



Figure 16 (continued)

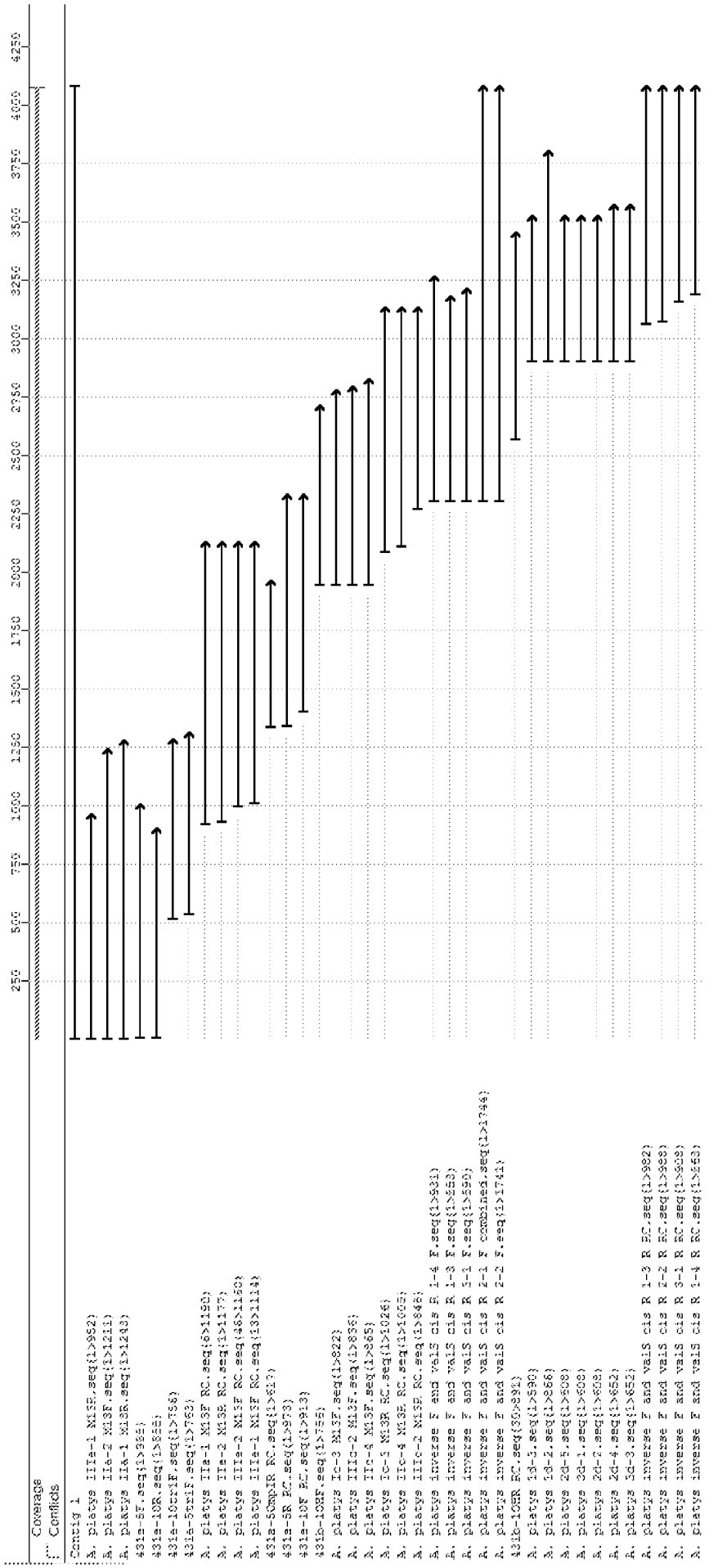

Coverage
Conflicts
Contig 1
A. platys IIIa-1 M13R.seq(1>952)
A. platys IIa-2 M13F.seq(1>1211)
A. platys IIa-1 M13R.seq(1>1243)
431a-5F.seq(1>965)
431a-10R.seq(1>888)
431a-10crsF.seq(1>756)
431a-5crsF.seq(1>763)
A. platys IIa-1 M13F RC.seq(6>1195)
A. platys IIa-2 M13F RC.seq(1>1177)
A. platys IIIa-2 M13F RC.seq(48>1158)
A. platys IIIa-1 M13F RC.seq(13>1114)
431a-5CmplR RC.seq(1>821)
431a-5R RC.seq(1>973)
431a-10F RC.seq(1>913)
431b-10GF.seq(1>755)
A. platys Ic-3 M13F.seq(1>822)
A. platys IIIa-2 M13F.seq(1>836)
A. platys IIc-4 M13F.seq(1>865)
A. platys Ic-3 M13R RC.seq(1>1026)
A. platys IIc-4 M13R RC.seq(1>1005)
A. platys IIIc-2 M13F RC.seq(1>846)
A. platys inverse F and vals cis R 1-4 F.seq(1>931)
A. platys inverse F and vals cis R 1-3 F.seq(1>853)
A. platys inverse F and vals cis R 3-1 F.seq(1>859)
A. platys inverse F and vals cis R 2-1 F combined.seq(1>1744)
A. platys inverse F and vals cis R 2-2 F.seq(1>1741)
431b-10GR RC.seq(30>838)
A. platys 1d-3.seq(1>896)
A. platys 1d-2.seq(1>886)
A. platys 2d-5.seq(1>908)
A. platys 3d-1.seq(1>908)
A. platys 2d-2.seq(1>908)
A. platys 2d-4.seq(1>852)
A. platys 3d-3.seq(1>852)
A. platys inverse F and vals cis R 1-3 R RC.seq(1>982)
A. platys inverse F and vals cis R 2-2 R RC.seq(1>988)
A. platys inverse F and vals cis R 3-1 R RC.seq(1>908)
A. platys inverse F and vals cis R 1-4 R RC.seq(1>853)
250
500
750
1000
1250
1500
1750
2000
2250
2500
2750
3000
3250
3500
3750
4000
4250

Figure 17

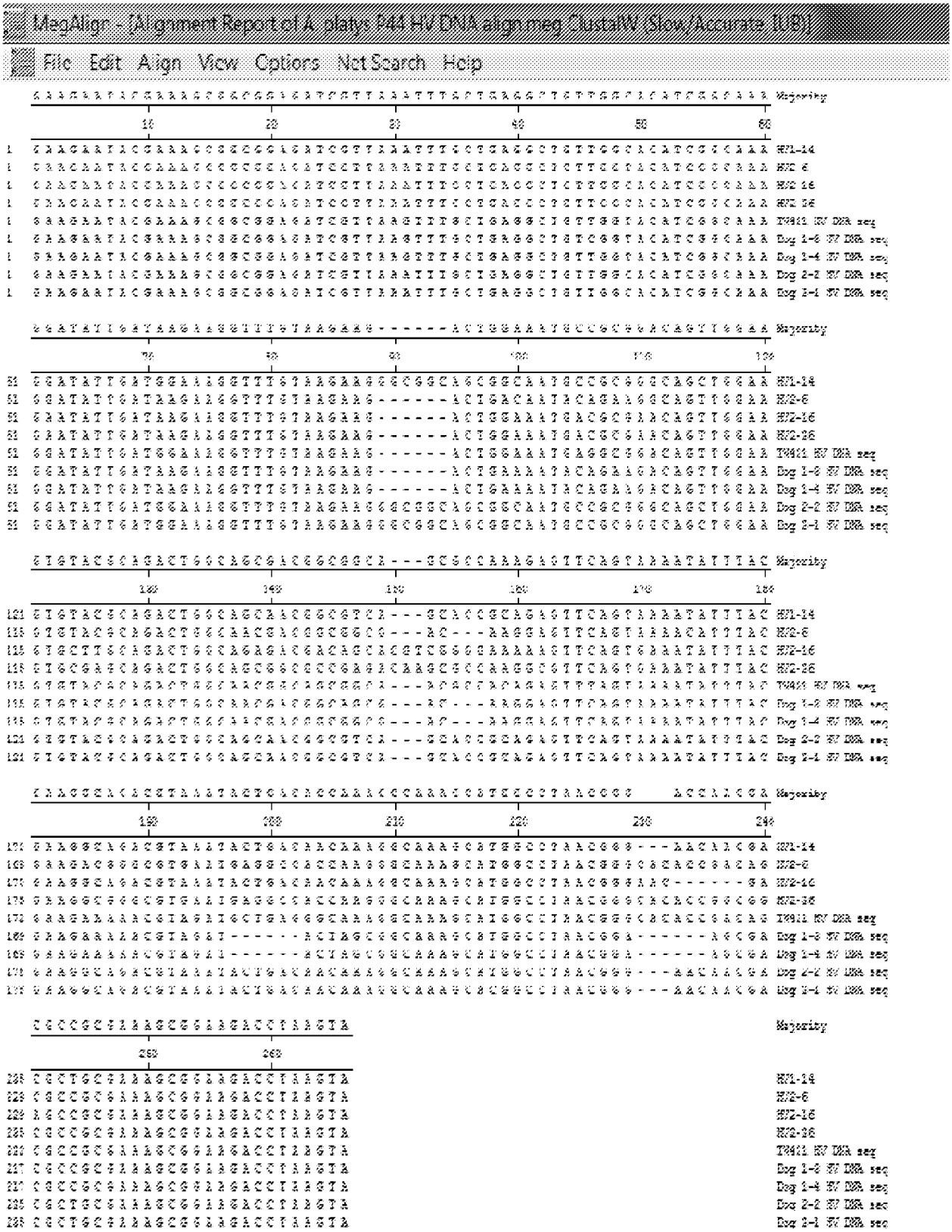

MegAlign - [Alignment Report of A. platy: P44 HV DNA align.meg ClustalW (Slow/Accurate) (UPI)]
File Edit Align View Options Net Search Help
Majority
HV1-14
HV2-6
HV2-16
HV2-26
TV411 HV DNA seq
Dog 1-2 HV DNA seq
Dog 1-4 HV DNA seq
Dog 2-2 HV DNA seq
Dog 2-1 HV DNA seq

Figure 18

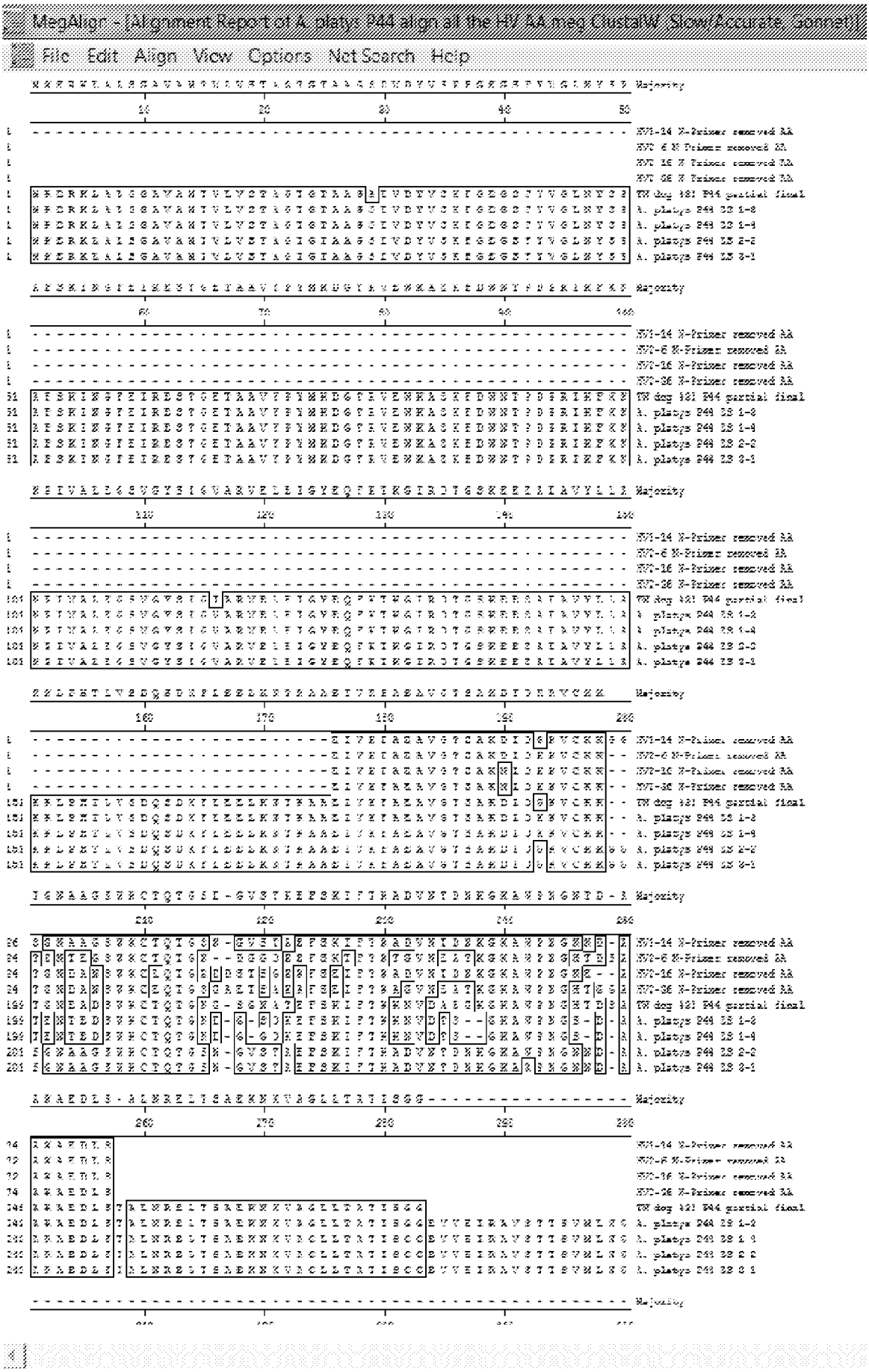



Figure 18 (continued)

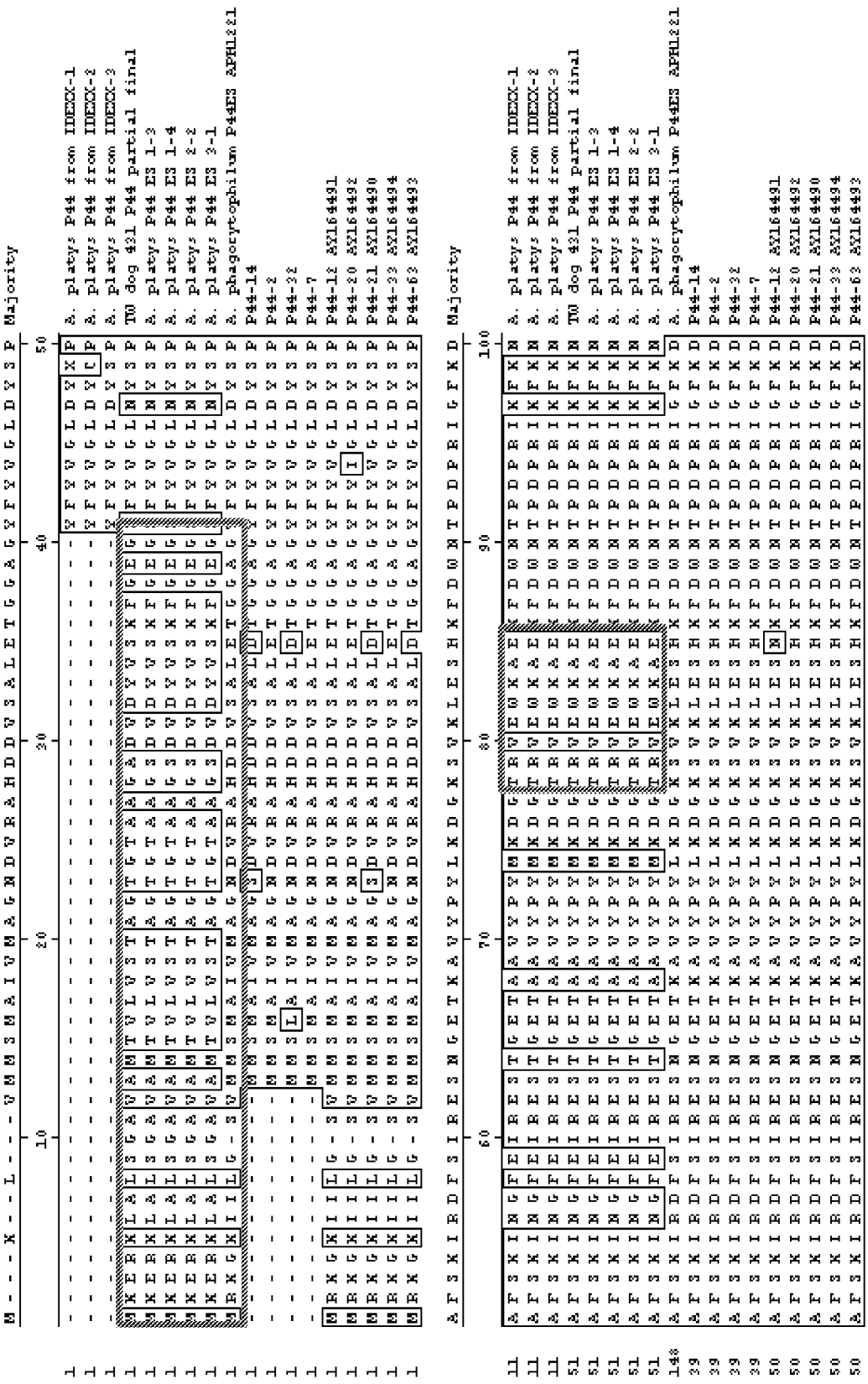


Figure 19

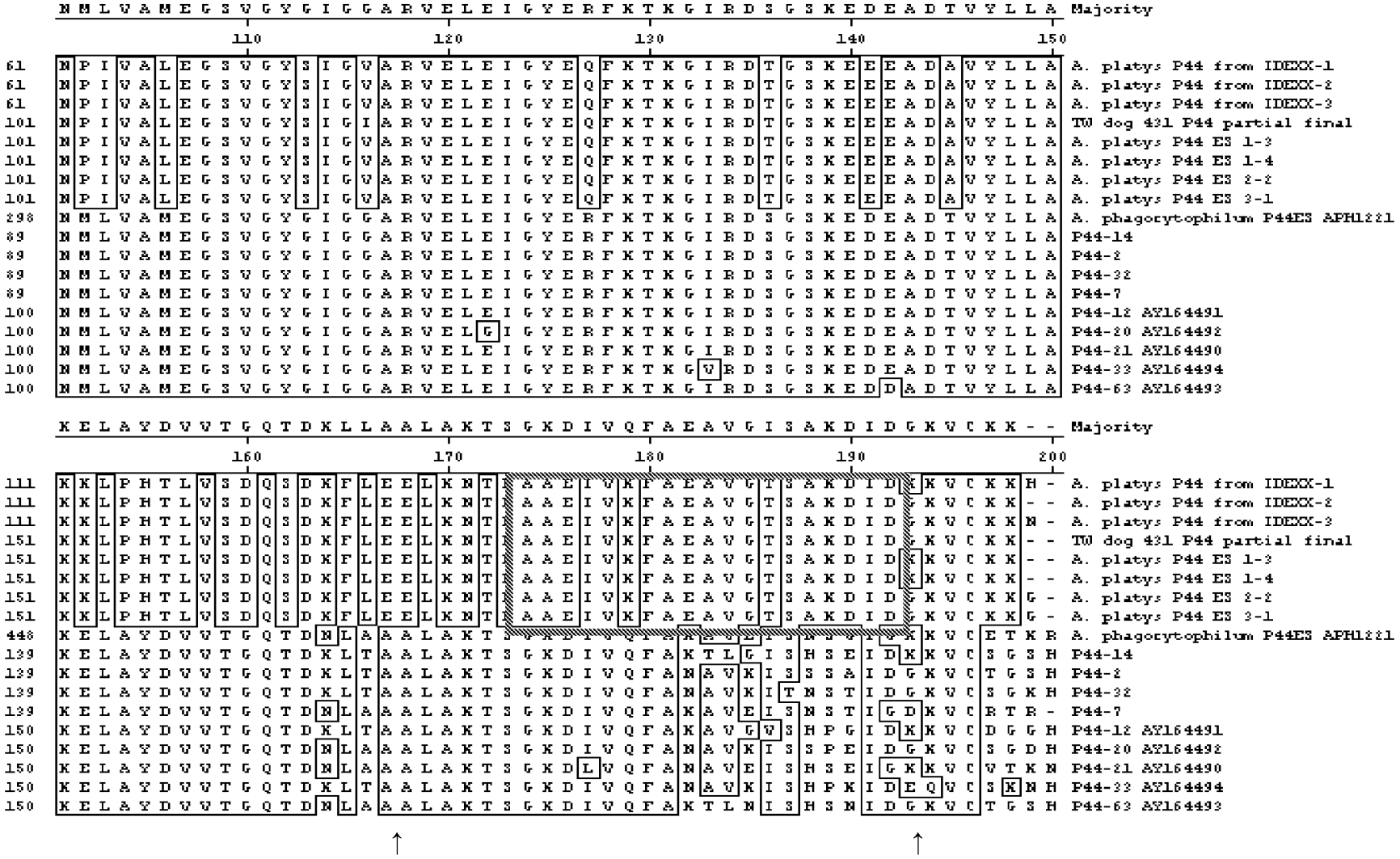



**Figure 19 (continued)**

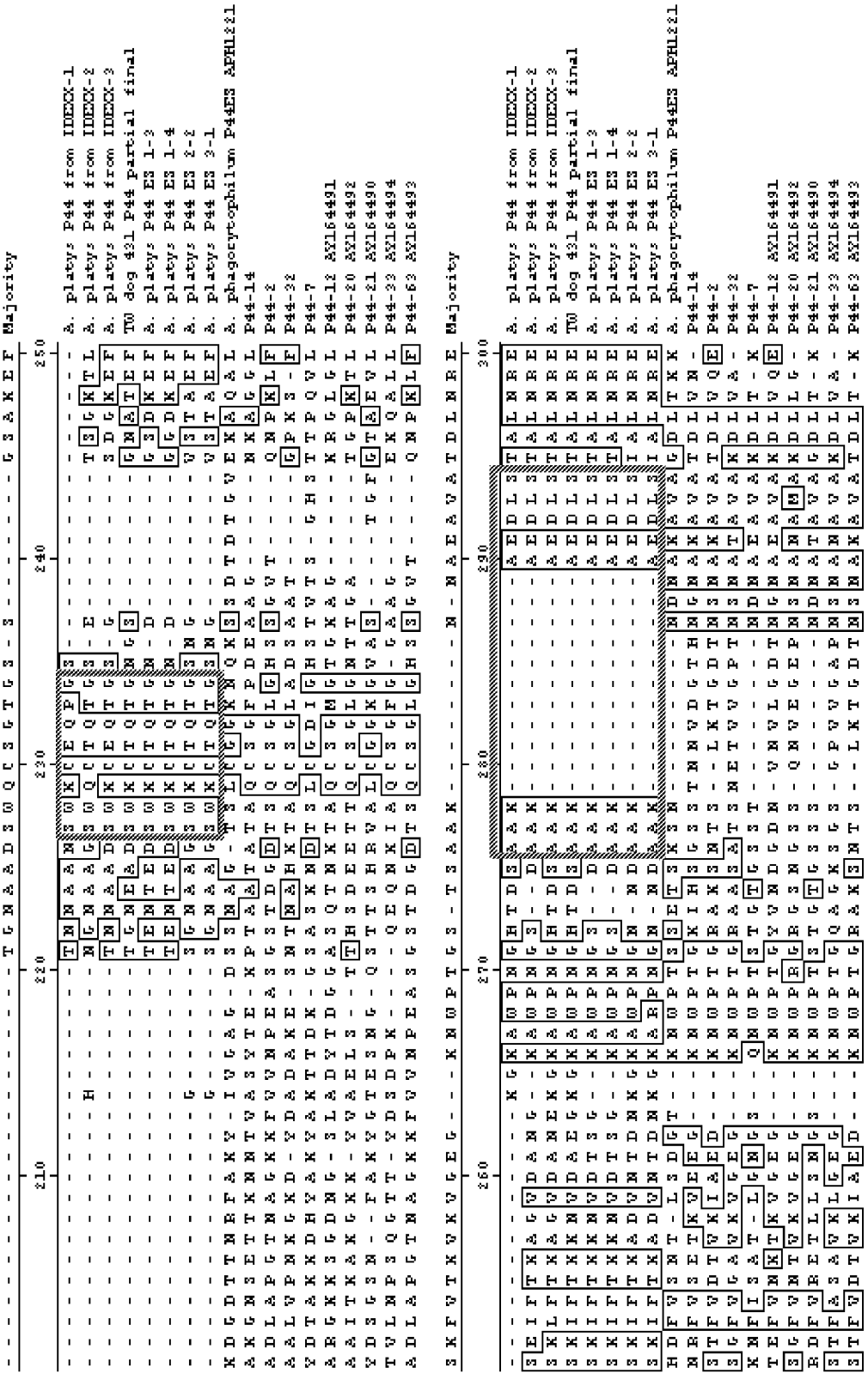



Figure 19 (continued)

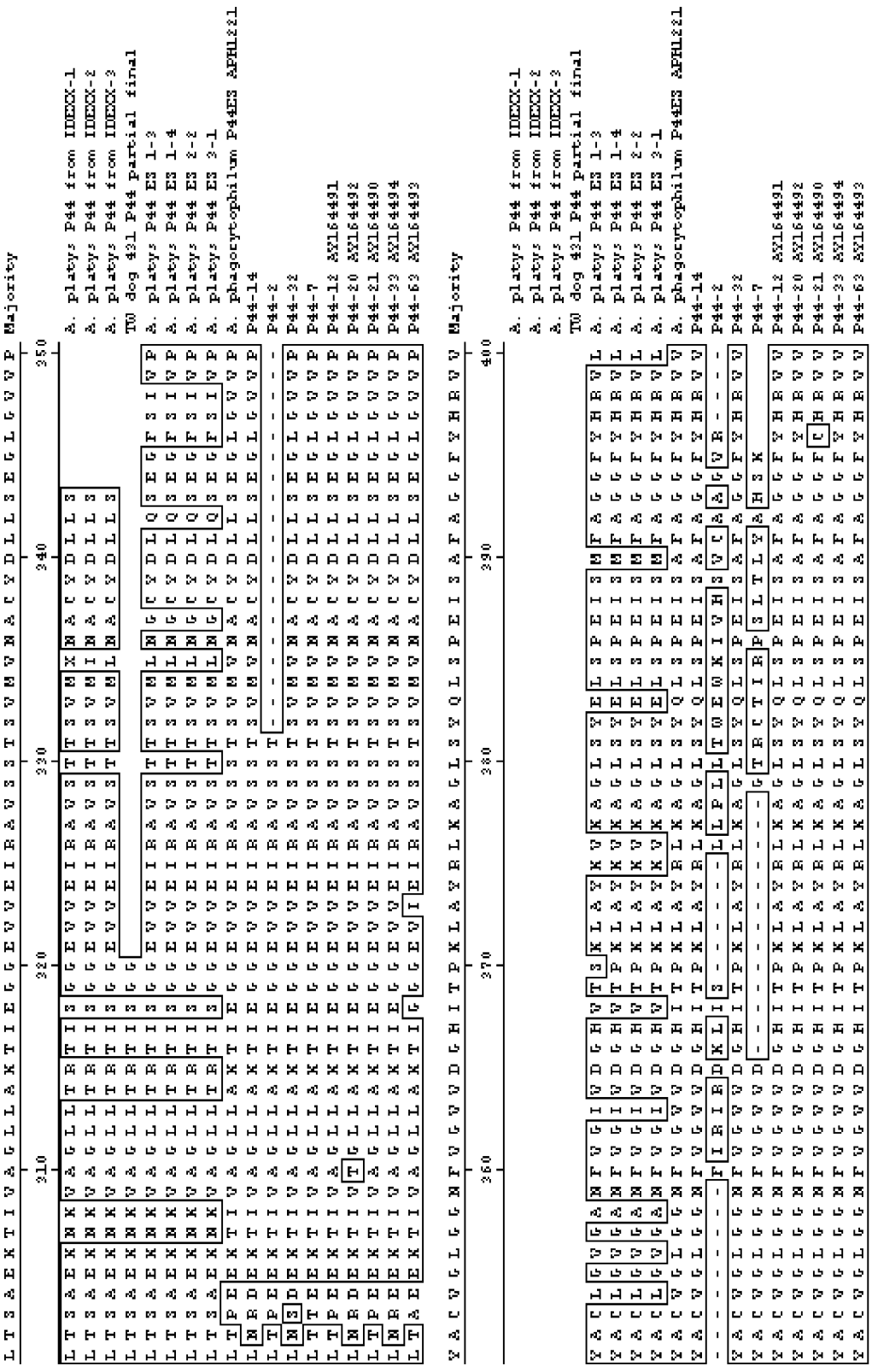



Figure 19 (continued)

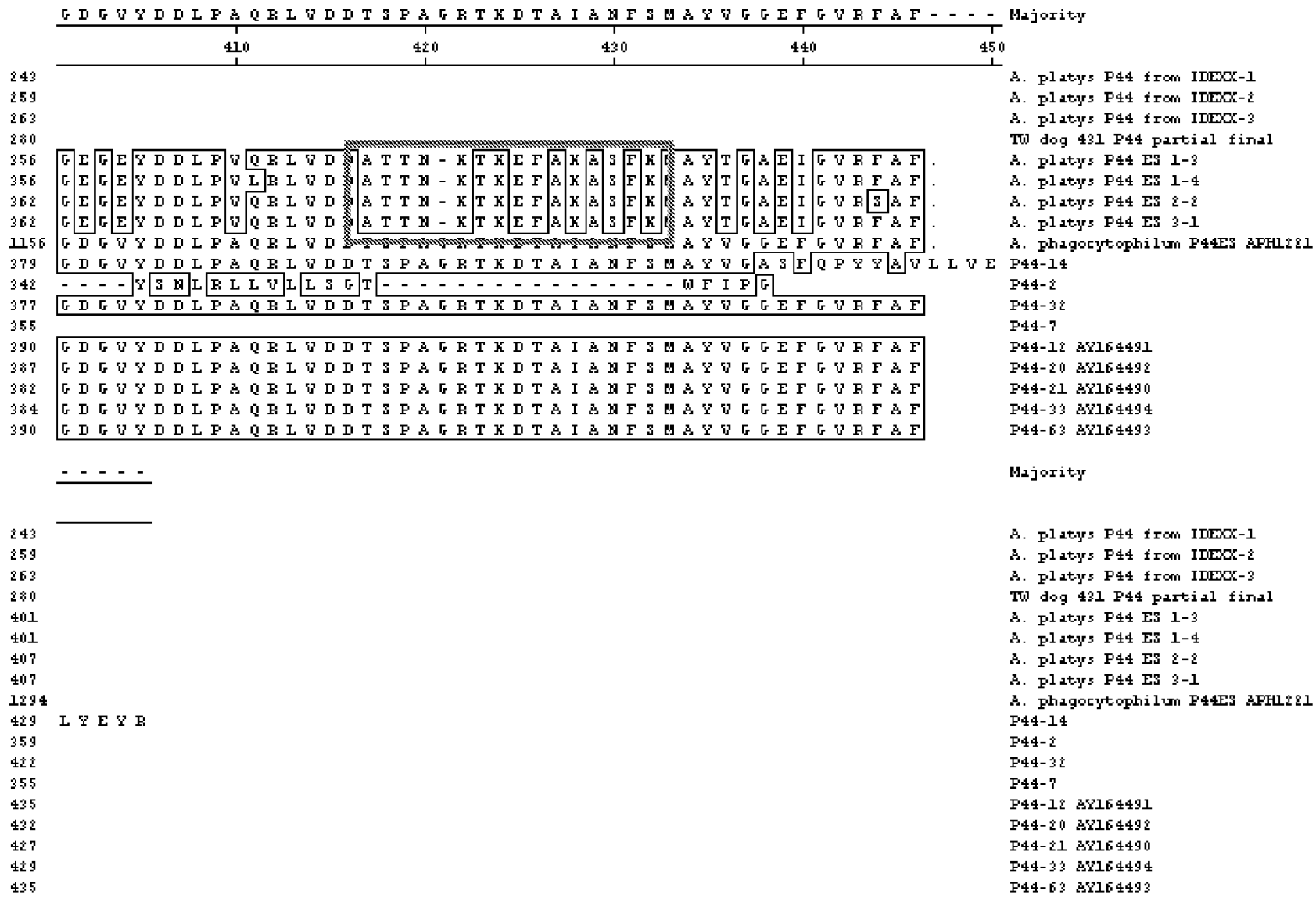



Figure 19 (continued)

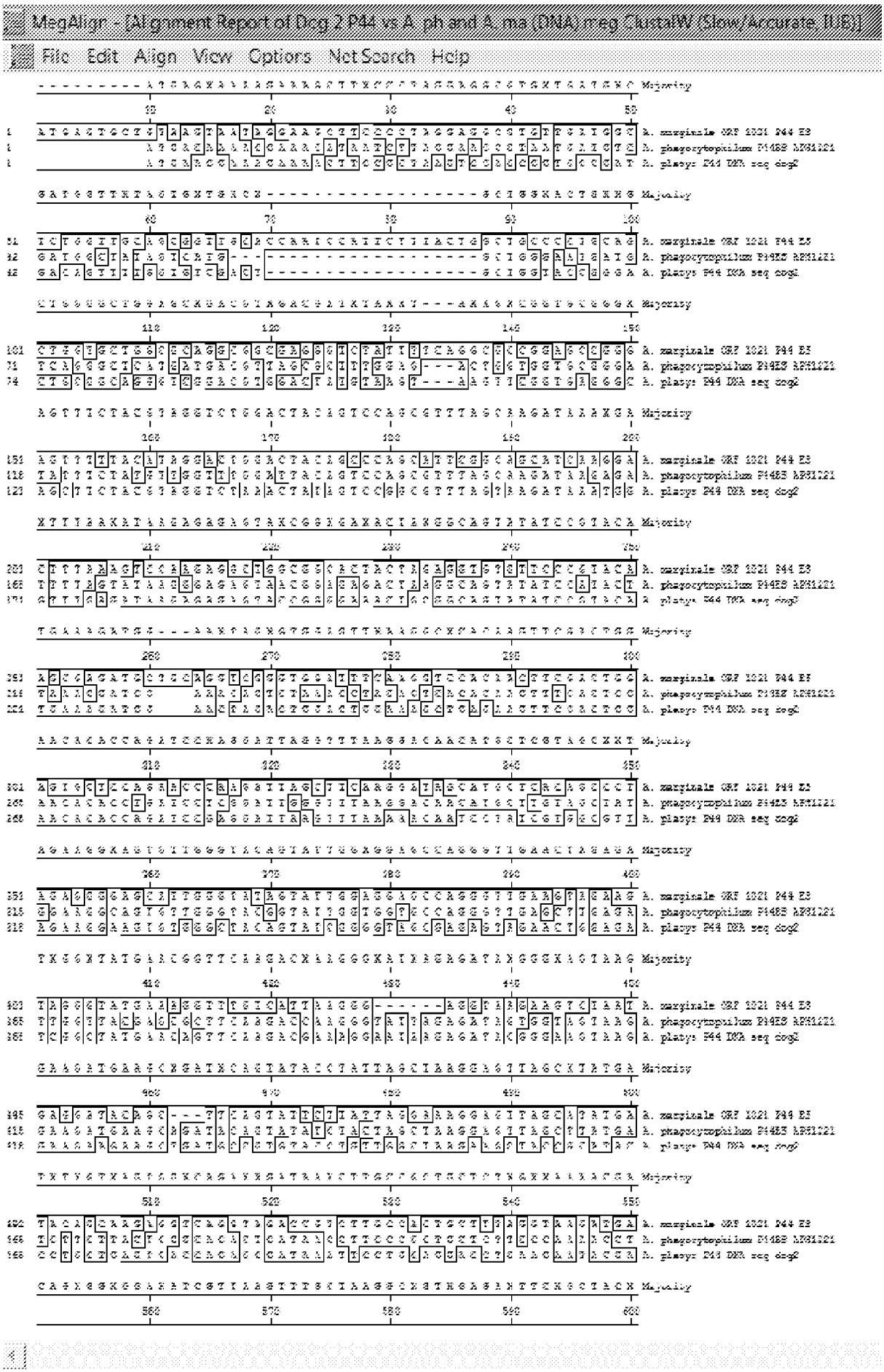

MegAlign - [Alignment Report of Dcg 2 P44 vs A. ph and A. ma (DNA) meg ClustalW (Slow/Accurate, IUB)]
File Edit Align View Options NotSearch Help

Figure 2n

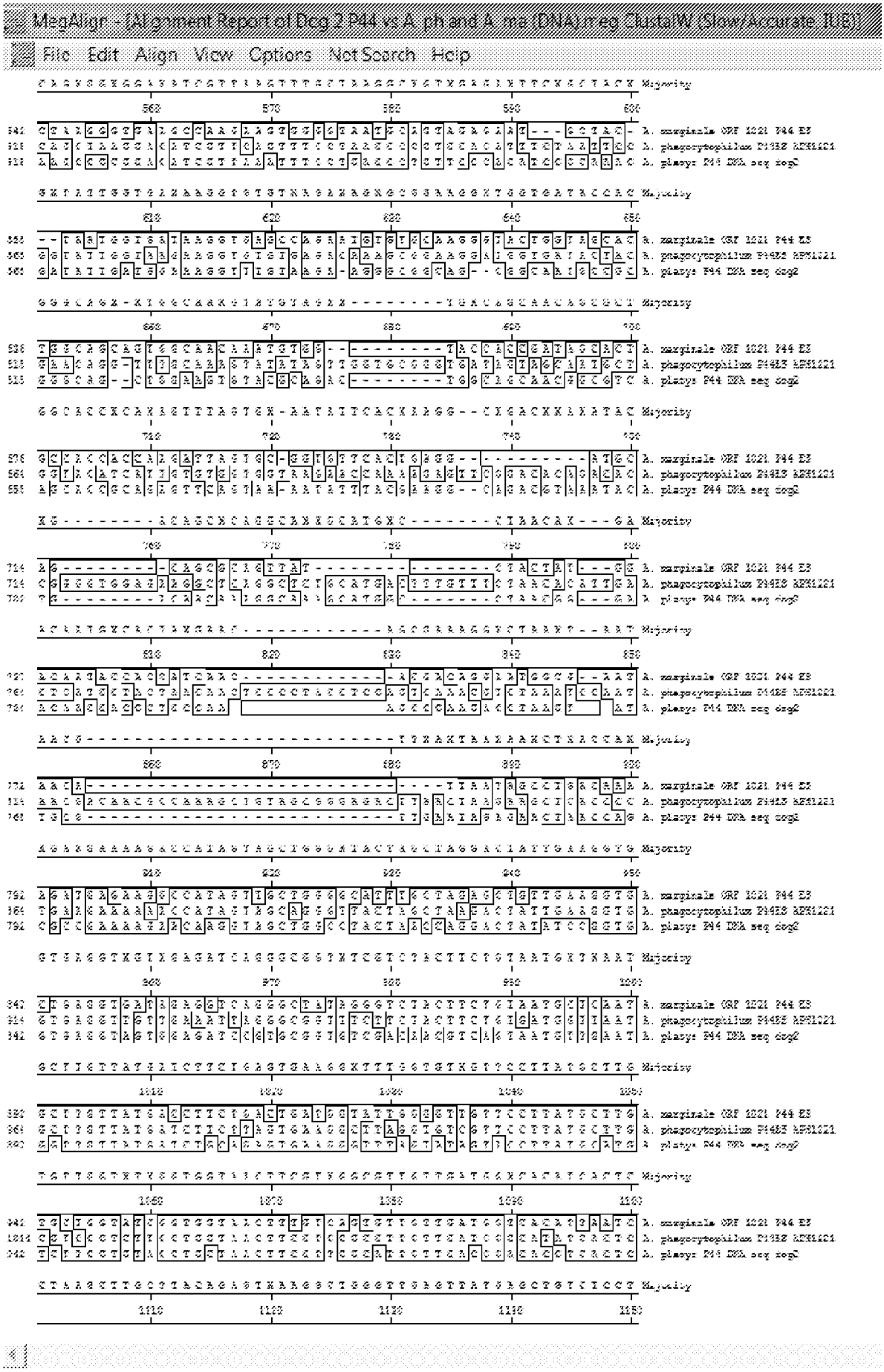



Figure 20 (continued)

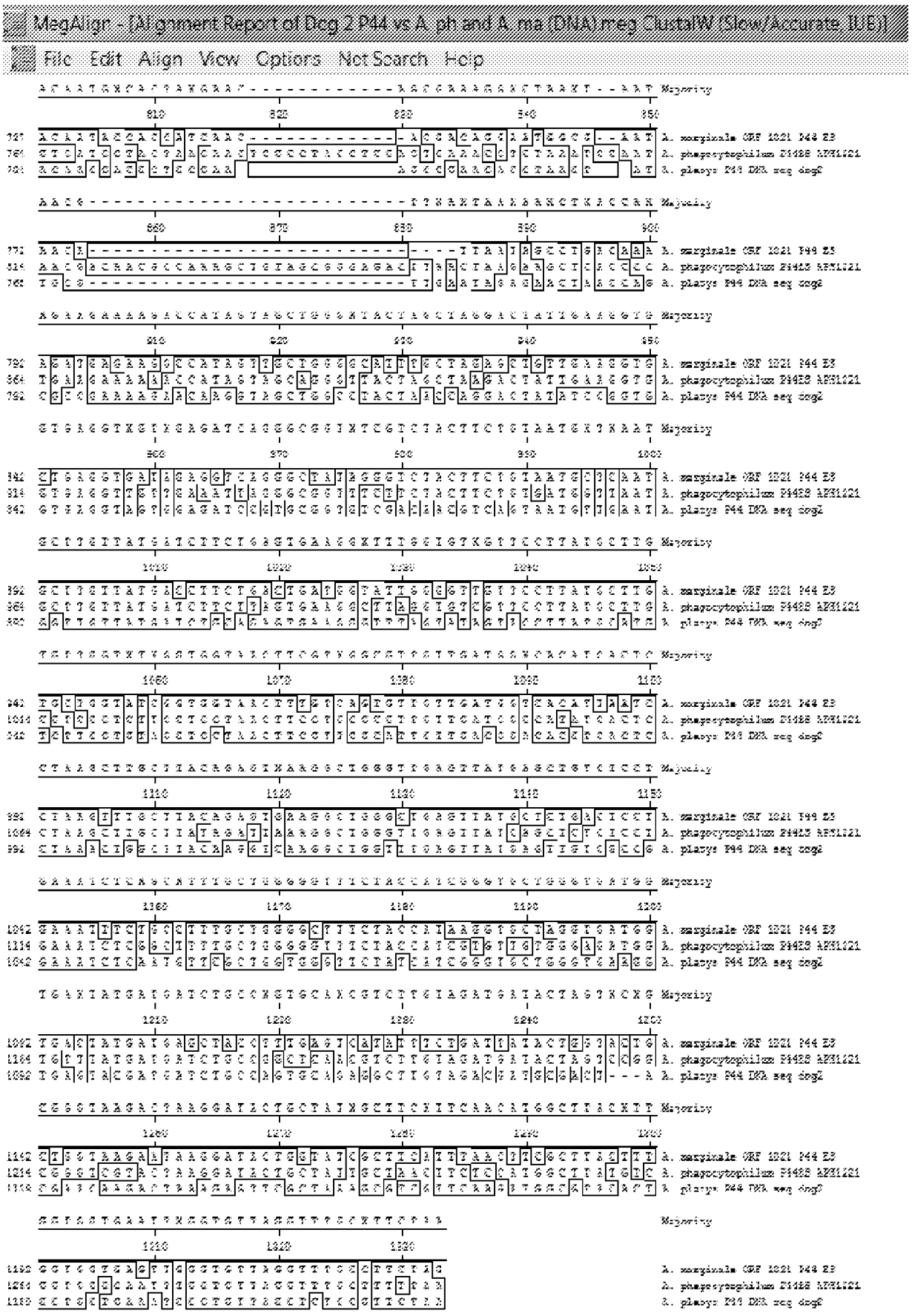



Figure 20 (continued)

# Figure 21

# Figure 21 (continued)

Figure 21 (continued)

Figure 21 (continued)

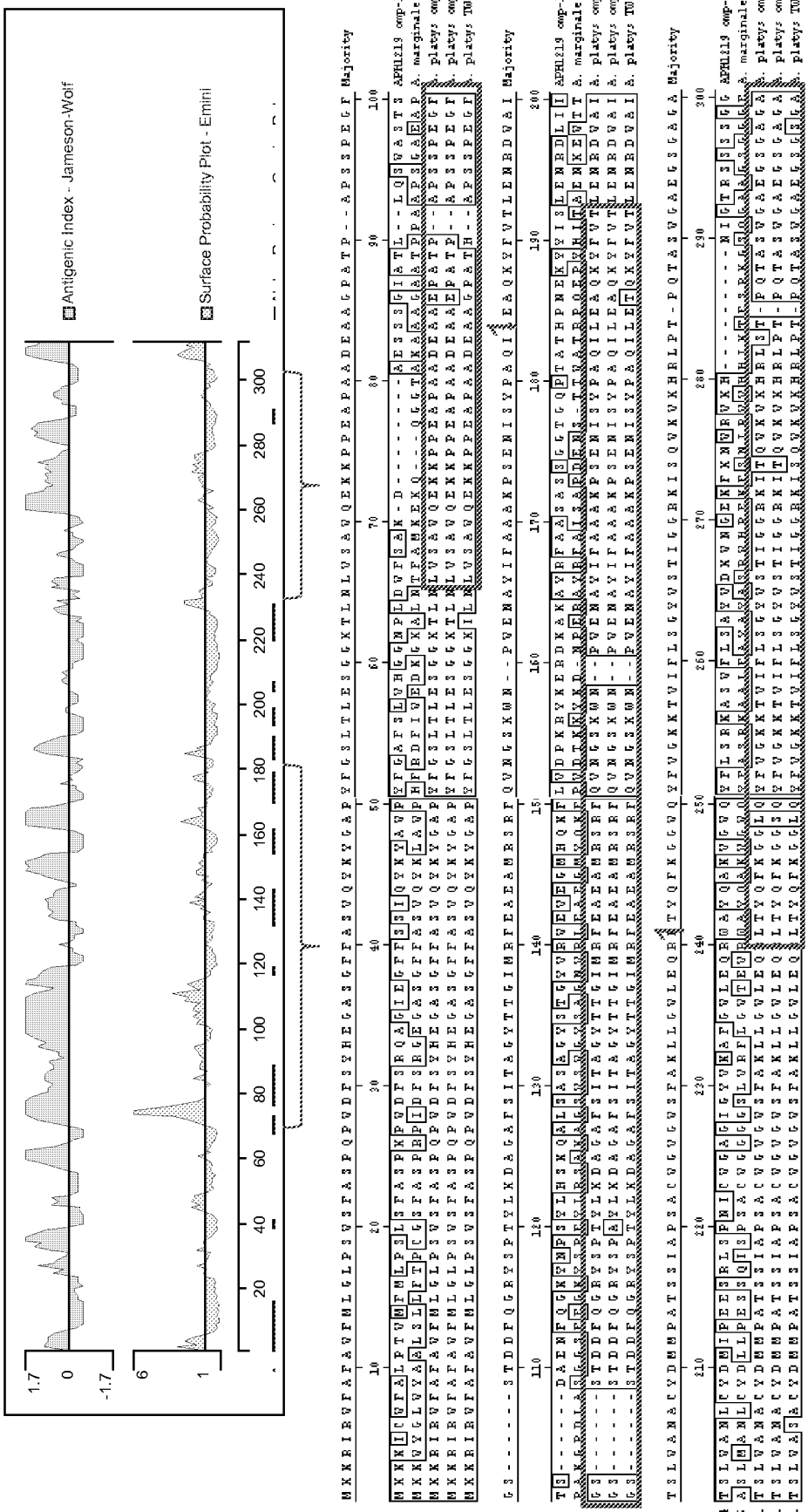

A. platys Omp1-x
Antigenic Index - Jameson-Wolf
Surface Probability Plot - Emini
Majority
APHL119 omp-LX aa seq
A. marginale omp-LX aa seq
A. platys omp-LX aa seq from dog 2
A. platys omp-LX aa seq from dog 3
A. platys TW dog #31 ompl-x AA seq

Figure 22

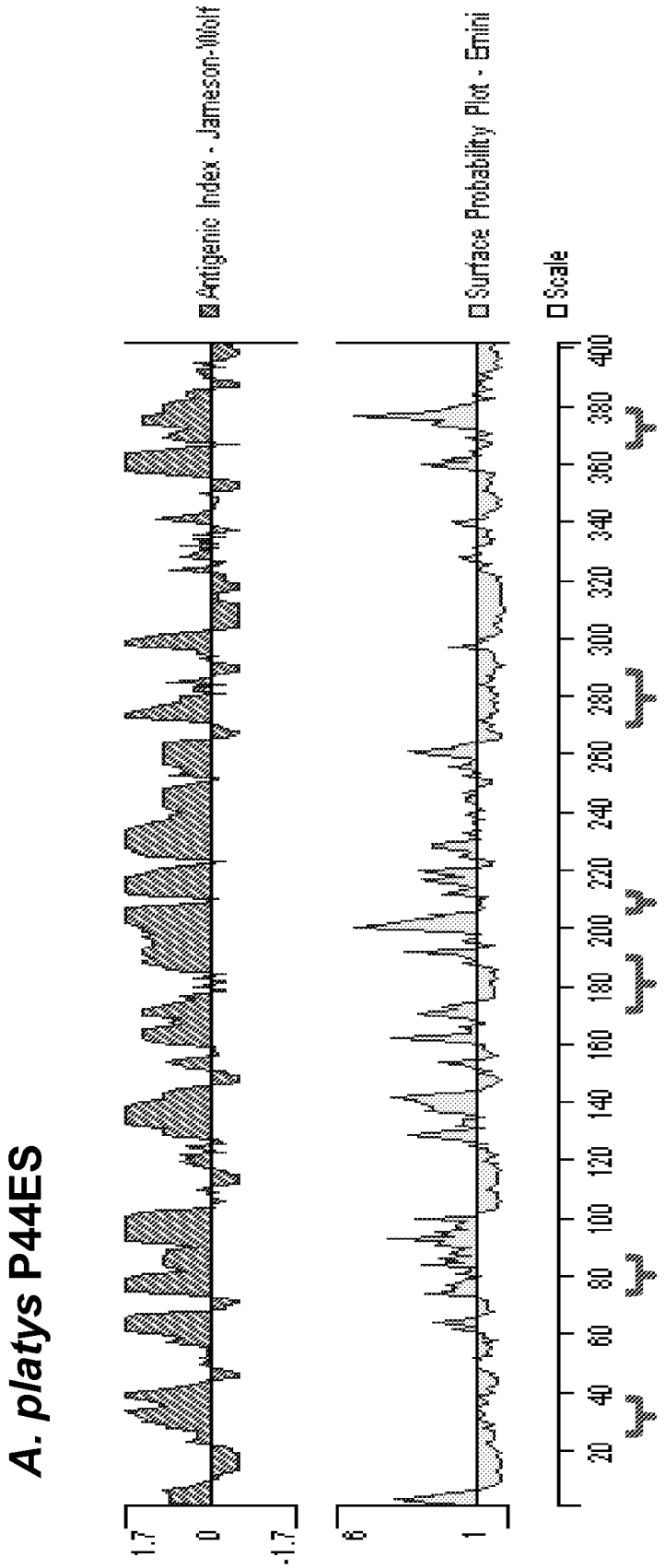

A. platys P44ES
Antigenic Index - Jameson-Wolf
Surface Probability Plot - Emini
Scale
1.7
0
-1.7
6
1
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400

Figure 23

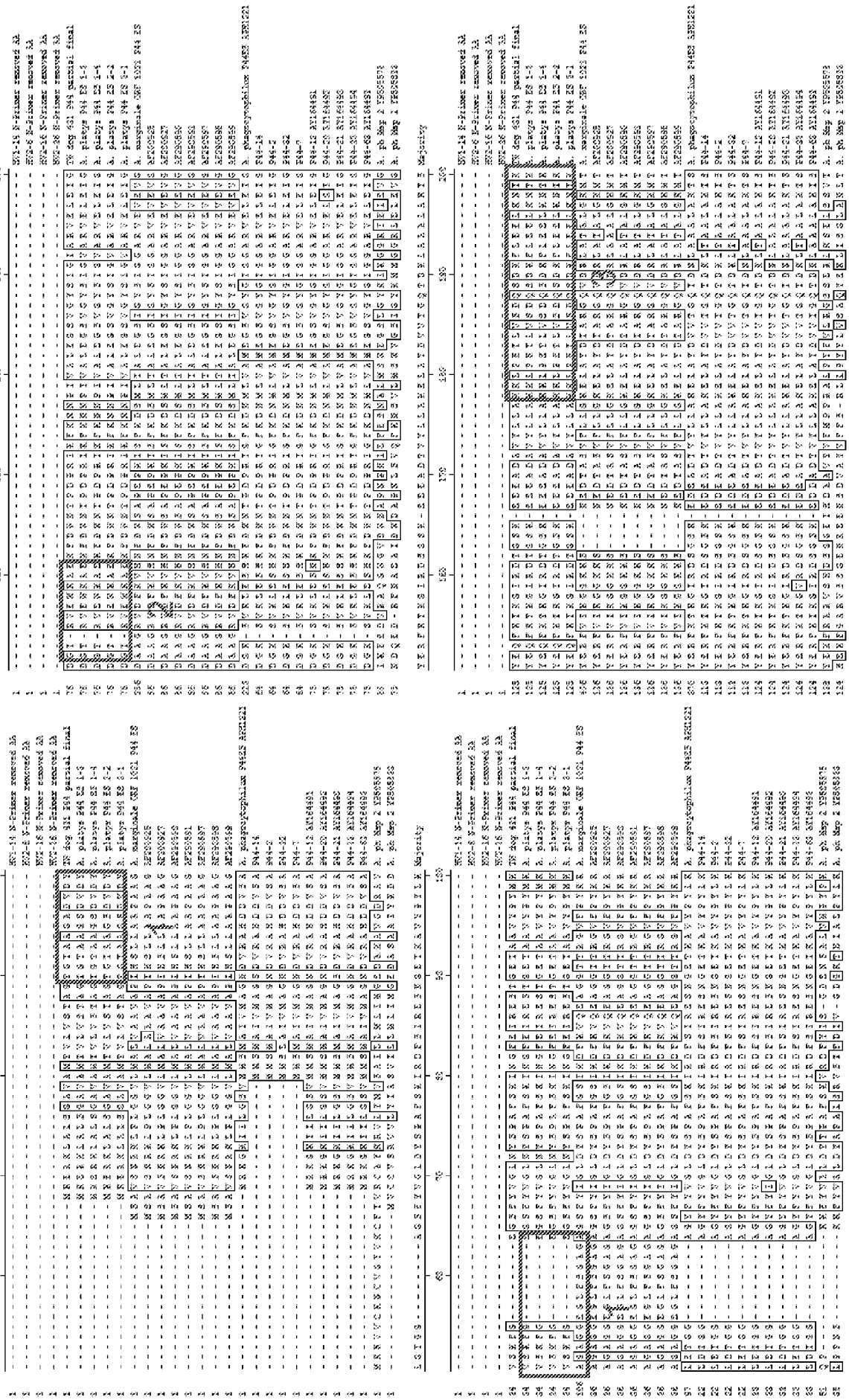


Figure 23 (continued)

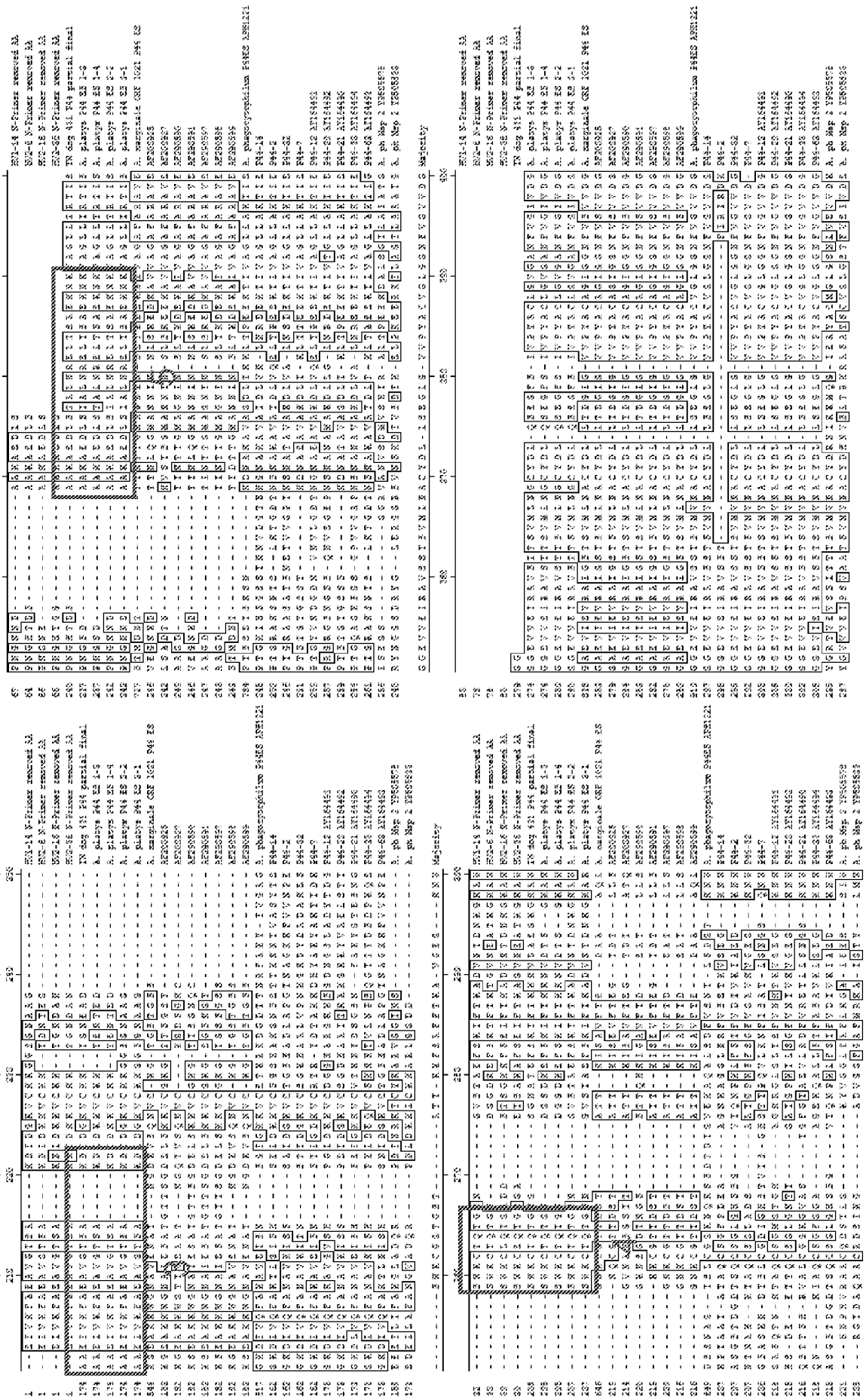


Figure 23 (continued)

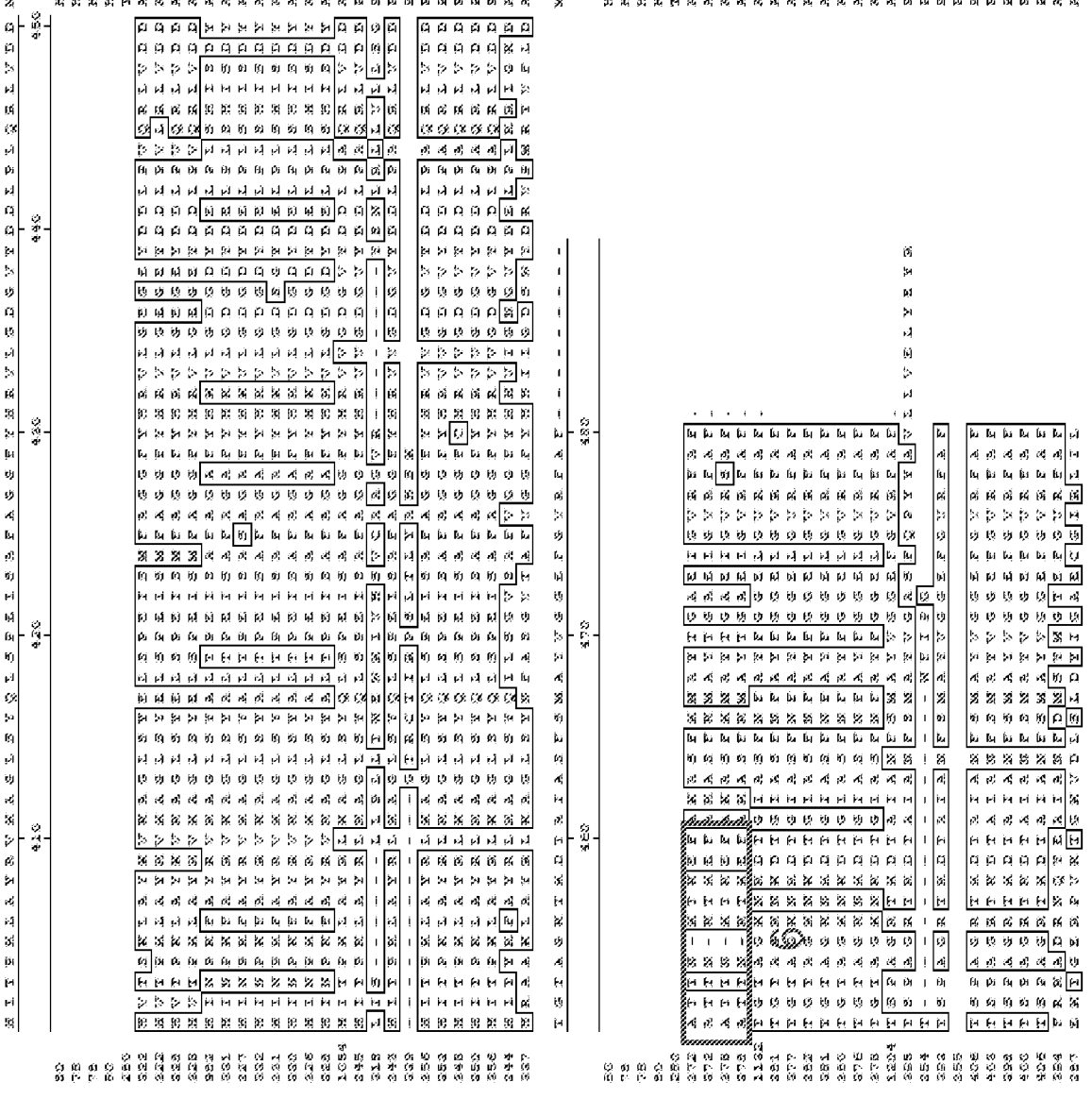


Figure 23 (continued)