CHAPTER 2

UREAPLASMA GENOTYPING

Fanrong Kong, Zhenfang Ma, Gregory James, Susannah Gordon, Gwendolyn L. Gilbert

Centre for Infectious Diseases and Microbiology, Insititute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, NSW 2145, Australia

This chapter has been published in *International Journal of Systematic and Evolutionary Microbiology* 2000; 50:1921-1929. (see Appendices section)

Statement of Joint Authorship

Kong, F. (candidate)
Did all the molecular work, interpreted the data and wrote the manuscript.
Ma, Z.
Assisted in some PCR.
James, G.
Provided all the needed molecular equipment, reagents and software.
Gordon, S.
Assisted in culture of related reference strains.
Gilbert G. L. (supervisor)
Supervised the overall project, assisted in research design, analysis and interpretation of data, and made a significant contribution to the manuscript.

2.1. SUMMARY

U. parvum comprises four serovars (1, 3, 6, 14) and *U. urealyticum*, ten serovars (2, 4, 5, 7-13). The multiple banded antigen genes (*mba*) of ureaplasmas contain both species and serovar specific sequences. To further elucidate the relationships between serovars and establish serovar identification assays, we sequenced the 5'-ends (including partial repetitive regions) and upstream regions of *mba* for all 14 serovars of ureaplasmas. All four serovars of *U. parvum* were clearly differentiated from each other. Ten serovars of *U. urealyticum* were divided into five *mba* genotypes, as follows: *mba* genotype A comprises serovars 2, 5, 8; *mba* genotype B, serovar 10 only; *mba* genotype C, serovars 4, 12, 13; *mba* genotype D, serovar 9 only; and *mba* genotype E comprises serovars 7 and 11. There were no sequence differences between members within each *mba* genotype. Further work is required to identify other genes or sequences in other regions of *mba* that may be used to differentiate *U. urealyticum* serovars within *mba* genotypes A, C and E. A better understanding of the molecular basis of serovar differentiation will help to improve serovar identification methods for use in studies of the pathogenesis and epidemiology of human ureaplasmas.

2.2. INTRODUCTION

Human ureaplasmas are recognised causes of urethritis (Taylor-Robinson *et al.*, 1985), and have been associated with complications of pregnancy and prematurity (Cassell *et al.*, 1988; Kundsin *et al.*, 1996). However, as common genital tract commensals, their pathogenic roles in individual cases are difficult to confirm (Robertson & Stemke, 1982; Heggie *et al.*, 2001).

U. parvum comprises four serovars (1, 3, 6, 14) and *U. urealyticum*, ten serovars (2, 4, 5, 7-13) (Robertson & Stemke, 1982). The relationships between serovars and disease syndromes needs to be studied further (Naessens *et al.*, 1988; Knox *et al.*,

2003). However, this has been limited by technical difficulties and cross-reactions associated with serotyping (Stemke & Robertson, 1985), even when monoclonal antibodies were used (Naessens *et al*., 1998).

Better understanding of the genetic basis of conventional ureaplasma serotyping will assist in development of a practical molecular serovar identification assay and allow further investigation of the pathogenic potential of individual serovars (Robertson & Stemke, 1982; Kong *et al*., 1999a). In our previous study, we showed that the sequences of the 16S rRNA genes and 16S-23S rRNA intergenic spacer regions, the urease gene subunits and adjoining regions were generally conserved for serovars within the two ureaplasma species. Only the 5'-ends (including partial repetitive regions) and upstream regions of *mba* showed heterogeneity between the 4 serovars of *U. parvum* and the 10 serovars of *U. urealyticum* (Kong *et al*., 1999b).

It has been suggested, previously, that the repetitive regions of the *mba* would contain serovar-specific sites (Watson *et al*., 1990; Zheng *et al*., 1996). In this study, we sequenced the 5'-ends of *mba* and upstream regions of all 14 serovars of ureaplasmas to determine whether these genes could be used to develop alternative serovar identification methods (Kong *et al*., 1999a, 2000).

2.3. MATERIALS AND METHODS

2.3.1. Bacterial strains.

Two sets of reference strains of all 14 serovars of ureaplasmas were used as previously described (Kong *et al*., 1999b) and as listed in chapter 1. One set was obtained directly from the American Type Culture Collection (ATCC reference strains) and the other was kindly provided by Dr. H. L. Watson, Department of Microbiology, University of Alabama at Birmingham, Alabama (UAB reference strains).

2.3.2. Oligonucleotide primers.

The oligonucleotide primers used in this study are shown in Table 2.1. Previously published oligonucleotide primers UMS-125, UMA226, UMA1213, UMA1586 (Teng *et al*., 1994; Zheng *et al*., 1995), and new primers designed by us – UMS-57, UMA222, UMSPS1, UMSPS2, UMAUA – based on previously published sequences (Zheng *et al.*, 1999 submitted to GenBank, accession numbers: U50459, U50460, U50461) (Zheng *et al*., 1995) were used to sequence *mba* of the four *U. parvum* serovars. Previously published oligonucleotide primers UMS-170, UMA226, UMA263 (Teng *et al*., 1994, 1995), and new primers designed by us – UMS-61, UMSUS, UMSUS1, UMSUS2, UMAUA, UMAUA1, UMAUA2 – based on the previously published sequences (Zheng *et al.*, 1999 submitted to GenBank, accession numbers: U50459, U50460, U50461) (Zheng *et al*., 1995) were used to sequence *mba* of all the ten *U. urealyticum* serovars.

Additional new primers – UMS3S, UMA314A, UMA314A', UMS14S, UMA1A, UMA1A', UMA6A, UMA6A' – based on sequences determined in this and previous studies (Kong *et al*., 1999b), and previously published primers designed by us UMS-83, UMS-54, UMA269, and UMA269' were designed specifically to amplify and differentiate *mba* of four *U. parvum* serovars 3, 14, 1 and 6. New primers UMA2A1, UMA2A2; UMA2A; UMA10A; UMA4A1, UMA4A2; UMA9A1, UMA9A2; UMA7A1, UMA7A2 and UMA7A3; UMS-112, UMS-112', UMA194, UMA194', UMA219 based on the sequences obtained in this study were designed to amplify and differentiate *mba* genotypes A to E (Table 2.1.).

2.3.3. DNA preparation and PCR.

DNA preparation and PCR system were used as previously described (Kong *et al*., 1999a, b).

Table 2.1. Primers for ureaplasma genotyping.

Primer	Specificity[a]	*T*m °C[b]	GenBank numbers	Sequence[c]
UMS-125[d] UMS-170[e]	Ureaplasmas	60.5	L20329	**1**GTA TTT GCA ATC TTT ATA TGT TTT CG**26**
UMA226[d]	"	64.0	"	**403**CAG/A CTG ATG TAA G/TTG CAG/A CAT TA/GA ATT C**376**
UMS-57	*U. parvum*	59.9	"	**64**GATTA/GA/C T/CAA ATC TTA GTG TTC ATA TTT TTT AC**95**
UMA222	"	63.6	"	**396**T GTA AGT GCA GCA TTA AAT TCA ATG**372**
UMS-61	*U. urealyticum*	61.2	AF055366	**103**GAAAAATA TTT GCA AAA CTA TAA ATA GAC AC**135**
UMA263[e]	"	70.8	"	**485**AAGTGACCT TTT GTT GTT GCG TTT TCT G**458**
UMSPS1	"	64.2	L20329	**469**CCT CGT GAA CCA AAA CCT AAT G**490**
UMSPS2	"	61.5	"	**517**GGA TTA ATC AAG ACT TCA GGT TTG**540**
UMA1213	UP 3/14	56.0	"	**1389**CTA AAG TAA TTA TTT TCC AGT AGT TTC**1363**
UMA1586	"	60.1	AE002134	**8480**GAT AAT CAT TCA TCT TCT CTT AAT TGT C**8507**
UMS3S	UP 3	58.9	L20329	**38**AATTAA TTA CTG TAG AAA TTA TGT AAG ATT ACC**70**
UMS14S	UP 14	58.9	AF056982	**36**AAAATT AAT TAC TGT AGA AAT TAT GTA AGA TTA AT**70**
UMA269	UP 3/14	61.7	L20329	**445**C AAC TAA ATG ACC TTT TTC AAG TGT AC**419**
UMA314A	"	60.4	"	**613**GTT GTT CTT TAC CTG GTT GTG TAG**590**
UMA314A'	"	63.2	"	**615**TG/TG TTG TTC TTT ACC TGG TTG TGT A**591**
UMS-54	UP 6	58.2	AF056984	**66**TTAATA AAT CTT AGT GTT CAT ATT TTT TAC TAG**98**

UMA6A	"	65.8	"	**615**CCT GGT TCT TGA GTT TTC GGA G**594**
UMA6A'	"	65.9	"	**619**TTT ACC TGG TTC TTG AGT TTT CGG**596**
UMS-83	UP 1	60.2	AF056983	**38**AATTAA TTACT GTA GAA ATT ATG TAA GAT TGC**69**
UMA1A	"	65.1	"	**619**TTT CTT TTG GTT CTT CAG TTT TTG AAG**593**
UMA1A'	"	64.5	"	**621**ATT TTC TTT TGG TTC TTC AGT TTT TGA**595**
UMA269'	UP 1/6	63.0	"	**445**CA ACC AAA TGA CCT TTT GTA ACT AGA T**419**
UMSUS	*U. urealyticum*	64.0	AF055366	**358**GTT TAC GAC ATT GAA AAT TTC GAT G**382**
UMAUA	"	66.4	"	**666**GGG G/TA/TG TTG/T A/C/TAC CAC/T TG/TC CTG GTT**638**
UMSUS1	"	53.1	"	**573**AAC TGC ATC TC/TT AGC/T ATT ACC T**594**
UMSUS2	"	56.7	"	**592**CCT GAT AAT TTG/T AAT TAT CAA ACA G**616**
UMAUA1	"	63.1	U50459	**1687**GCC CAA TTC ATA GGC TAT TAA TTG**1664**
UMAUA2	"	63.8	"	**1696**AAA AAA ATA GCC CAA TTC ATA GGC**1673**
UMA2A1	UU A/B	64.2	AF055366	**646**TTC CTG GTT TTG TTT CAA AAC CTA T**622**
UMA2A2	"	64.4	"	**649**CAC TTC CTG GTT TTG TTT CAA AAC**626**
UMA2A	UU A	62.5	"	**674**CCA CTT CCT GGT TTT GTA GTT TC**652**
UMA10A	UU B	65.0	AF055358	**674**CCA CTT CCT GGT TGT GTA GTT GA**652**
UMA4A1	UU C	69.1	AF055363	**647**TT GCC TGG TTG TGT TTC GAA CTC**625**
UMA4A2	"	69.3	"	**649**CAT TGC CTG GTT GTG TTT CGA AC**627**
UMA9A1	UU D	68.3	AF055367	**655**CTG GAG TTG GTG TAG GCG CAT**635**

UMA9A2	"	68.4	"	**657**TTC TGG AGT TGG TGT AGG CGC**637**
UMA7A1	UU E	67.2	AF055365	**440**GTA ATT GCA ACA TGG AAT TCA GTT TCA**415**
UMA7A2	"	63.0	"	**653**GGT TCT GGT GTA TGA GTG CTT TT**631**
UMA7A3	"	63.3	"	**656**GTT GGT TCT GGT GTA TGA GTG C**635**
UMA219	UU A–D	70.3	AF055366	**440**GTA ATT GCA ACA TGG AAT TCA GCT TCG**414**
UMS-112	UU A/D/E	56.3	"	**59**GAT TAA ACA AAA TCT TAA TGT TGT TA**84**
UMA194	UU A/D/E	63.3	"	**415**CGT TTA ATG CTT TTT TAT CAT TTT CAG**389**
UMS-112'	UU B/C	58.6	AF055363	**59**GAT TAA ACA AAA TCT TAA TGT TGT TG**84**
UMA194'	UU B/C	62.8	"	**415**CGT TTA ATG CTT TTT TAT CAT TTT CAT**389**

Notes.

a. UP 3: *U. parvum* serovar 3; UP 14: *U. parvum* serovar 14; UP 3/14: *U. parvum* serovars 3 and 14; UP 1: *U. parvum* serovar 1; UP 1/ 6: *U. parvum* serovars 1 and 6. UP 6: *U. parvum* serovar 6. UU A: *U. urealyticum mba* genotype A, includes serovars 2, 5, and 8; UU B: *U. urealyticum mba* genotype B, includes serovar 10; UU C: *U. urealyticum mba* genotype C, includes serovars 4, 12, and 13; UU D: *U. urealyticum mba* genotype D, includes serovar 9; UU E: *U. urealyticum mba* genotype E, includes serovars 7 and 11.

b. Primer melting temperatures (*T*m) were provided by the primer synthesiser (Sigma-Aldrich, Castle Hill, NSW, Australia).

c. Numbers represent the numbered base positions at which primer sequences start and finish (numbering start point "1" refer to the start points "1" of correspondent gene GenBank accession numbers). Underlined sequences show bases added to modify previously published primers (Teng *et al*., 1994; Kong *et al*., 2000). Letters behind "/" indicate alternative nucleotides in different serovars.

d. From Teng, *et al.*, 1994.

e. From Teng, *et al.*, 1995.

To amplify the 5'-ends and repetitive regions of *mba* genes of *U. parvum* serovars for sequencing, a nested PCR was developed, using UMS-125-UMA1586 as outer primers and UMS-57-UMA1213 (for serovars 3 and 14) and UMS-57-UMAUA (for all four serovars of *U. parvum*) as inner primers. Nested PCR was also used to amplify the 5'-ends and repetitive regions of the *mba* of the ten *U. urealyticum* serovars for sequencing. The outer primers were UMS-170-UMAUA2 and inner primers were UMS-61-UMAUA and UMSUS-UMAUA2 (or UMSUS-UMAUA1) (Table 2.1.).

The denaturation, annealing and elongation temperatures and times used for the first step PCR were 95^{o}C for 30 seconds, 50^{o}C for 30 seconds and 72^{o}C for 3 minutes, respectively, for 30 cycles. For the second step PCR, the denaturation, annealing and elongation temperatures and times used were 95^{o}C for 30 seconds, 55^{o}C for 30 seconds and 72^{o}C for 2 minutes, for 30 cycles. For the serovar- or genotype-specific PCR, the denaturation, annealing and elongation temperatures and times used were 95^{o}C for 30 seconds, 55-62^{o}C (according to the *T*m value) for 30 seconds and 72^{o}C for 1 minute, respectively, for 40 cycles.

12.5 μl of PCR products were analysed by electrophoresis on 2.0% agarose gels, which were stained with 0.5 mg/ml ethidium bromide. For sequencing, PCR products of appropriate size that produced visible bands on ultraviolet illumination were further purified. For identification of individual subtypes, the presence of PCR amplicons of expected length on ultraviolet transillumination were accepted as positive.

2.3.4. Sequencing and sequence analysis.

The PCR products were sequenced using Applied Biosystems (ABI) *Taq* DyeDexoy terminator cycle-sequencing kits according to standard protocols. Primer UMSPS1 (or UMSPS2) was used as sequencing primer for the amplicons of UMS-57-UMA1213 (for serovars 3 and 14), and UMS-57-UMAUA (for serovars 1 and 6);

UMSUS, UMSUS1 (or UMSUS2) were used as sequencing primers for the amplicons of UMS-61-UMAUA, and UMSUS-UMAUA2 (or UMSUS-UMAUA1).

Multiple sequence alignments were performed using *Pileup* and *Pretty* programs from the Multiple Sequence Analysis program group, provided in WebANGIS, ANGIS (Australian National Genomic Information Service), 3rd version.

2.3.5. Nucleotide sequence accession numbers.

The new sequence data in the study were deposited into GenBank Nucleotide Sequence Databases with the following accession numbers: AF055358-AF055367, AF056982-AF056984 (*mba*). The following GenBank sequences were used as references: NC_002162 (*U. parvum* serovar 3 genome), U50462 (serovar 14 *mba*, partial coding sequences [cds]), L20329 (serovar 3 *mba*, complete cds), U50459 (serovar 10 *mba*, complete cds), U50460 (serovar 2 *mba*, partial cds) and U50461 (serovar 4 *mba*, partial cds).

2.4. RESULTS

2.4.1. PCR and sequencing.

As predicted, the inner primer pair UMS-57-UMA1213 produced amplicons only from serovars 3 and 14 and UMS-57-UMAUA produced amplicons from all four serovars of *U. parvum.* From *U. urealyticum,* inner primers UMS-61-UMAUA produced amplicons from all ten serovars whereas UMSUS-UMAUA2 (or UMSUS-UMAUA1) produced amplicons from seven (all except serovars 9, 7 and 11). UMS-125-UMA226 produced amplicons from all 14 serovars of *U. parvum* and *U. urealyticum.* The relevant portions of sequencing results for each serovar were submitted to GenBank after further analysis (see below).

2.4.2. Comparative study of the sequences of the 5'-ends and upstream of *mba*.

There were base differences at 45 (45/601=7.5 %) sites at the *mba* regions (1 ~ 650) among the four serovars of *U. parvum* (Kong *et al.*, 1999b) (Figure 2.1.). There were base differences at 22 (22/634=3.5%) sites at the *mba* regions (1 ~ 639) among the ten serovars of *U. urealyticum* (Figure 2.1.).

Nucleotide sequences of the *mba* repetitive units of *U. parvum* and *U. urealyticum* are shown in Figure 2.1. They begin in the vicinity of nucleotide 651. There were differences between sequences from all four *U. parvum* serovars. Sequences from serovars 2, 5 and 8 of *U. urealyticum* were identical and grouped as *mba* genotype A. The serovar 10 sequence was the same length but differed from *mba* genotype A by 3/24 nucleotide bases and it was classified as *mba* genotype B. Serovar 4, 12 and 13 sequences were longer than those of *mba* genotypes A and B, but identical with each other and were grouped together as *mba* genotype C. No repetitive units were identified for serovars 9, 7 and 11. However, there were differences between serovar 9 and serovars 7/11 in 58 (58/391=14.8%) nucleotide bases in the region 640-1033 and 14 (14/634=2.2%) in the region 1-639 (Figure 2.1.). These differences defined two additional *mba* genotypes, D (serovar 9) and E (serovars 7 and 11).

2.4.3. The specificity of *Ureaplasmas* genotyping primers.

All the ATCC and UAB reference strains of *U. parvum* and *U. urealyticum* were correctly identified by the *mba* serovar- or genotype-specific primer pairs. The PCR results for all the serovars of *U. parvum* and *U. urealyticum*, using the 36 serovar- or genotype-specific primer pairs to amplify the 5'-ends of the *mba* are summarised in Table 2.2.

Figure 2.1. Multiple sequence alignment of the 5'-end and upstream of *mba.*

```
           1                                                    50
 serovar 1  ---------- ---------- ---------- ---------- -----a--g-
 serovar 3  ---------- ---------- ---------- ---------- -----a--g-
serovar 14  ---------- ---------- ---------- ---------- -----a--g-
 serovar 6  ---------- ---------- ---------- ---------- -----a----
serovar 11  ---------- ---------- ---------- ------.... .---------
 serovar 7  ---------- ---------- ---------- ------.... .---------
 serovar 9  ---------- ---------- ---------- ------.... .---------
serovar 12  ---------- ---------- ---------- ------.... .---------
serovar 13  ---------- ---------- ---------- ------.... .---------
 serovar 4  ---------- ---------- ---------- ------.... .---------
 serovar 2  ---------- ---------- ---------- ------.... .---------
 serovar 5  ---------- ---------- ---------- ------.... .---------
 serovar 8  ---------- ---------- ---------- ------.... .---------
serovar 10  ---------- ---------- ---------- ------.... .---------
 Consensus  GTATTTGCAA TCTTTATATG TTTTCGTTAA AATTAAAAAT TAATTCCTAT

           51                                                   100
 serovar 1  -g---tt-tg -a-----..g -t-------- g----ca--- -t-t--c---
 serovar 3  -g---tt-tg -a-----..- -c-------- g----ca--- -t-t--c-t-
serovar 14  -g---tt-tg -a-----..- at-------- g----ca--- -t-t--c-t-
 serovar 6  -----tt-tg -a-----..- at-------- g----ca--- -t-t--ctag
serovar 11  ---------- ---------- ---------- ---------- ----------
 serovar 7  ---------- ---------- ---------- ---------- ----------
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- ---------- --------g- ----------
serovar 13  ---------- ---------- ---------- --------g- ----------
 serovar 4  ---------- ---------- ---------- --------g- ----------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- ---------- --------g- ----------
 Consensus  AAAAACAACA TGAGATTAAA CAAAATCTTA ATGTTGTTAT TATCTATACA

           101                                                  150
 serovar 1  -at----.t- --g..... .......... .......... ..........
 serovar 3  -at----.t- ---..... .......... .......... ..........
serovar 14  -at----.t- ---..... .......... .......... ..........
 serovar 6  -at----tt- ---..... .......... .......... ..........
serovar 11  ---------- ---------- ---------- ---------- ----------
 serovar 7  ---------- ---------- ---------- ---------- ----------
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- ---------- ---------- ----------
serovar 13  ---------- ---------- ---------- ---------- ----------
 serovar 4  ---------- ---------- ---------- ---------- ----------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- ---------- ---------- ----------
 Consensus  TTCTAAAGAA AAATATATTT GCAAAACTAT AAATAGACAC AAAAAACAAT

           151                                                  200
 serovar 1  .........c --ta----ga -------tt- a--------- -..a-c----
 serovar 3  .........c --ta----ga -------tt- a--------- -..a-c----
serovar 14  .........c --ta----ga -------tt- a--------- -..a-c----
 serovar 6  .........c --ta----ga -------tt- a--------- -..acc----
serovar 11  ---------- ---------- -g-------- ---------- ----------
 serovar 7  ---------- ---------- -g-------- ---------- ----------
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- -g-------- ---------- ----------
serovar 13  ---------- ---------- -g-------- ---------- ----------
 serovar 4  ---------- ---------- -g-------- ---------- ----------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- -g-------- ---------- ----------
 Consensus  AGAATAATAA AACTAAATTT CATATTTAGT TTATTAGGAG ATCGTTATAA
```

```
            201                                                 250
 serovar 1  ---------- ---------- a-----c--- --t--g---t ----a--t--
 serovar 3  ---------- ---------- a-----c--- --t--g---t ----t--t--
serovar 14  ---------- ---------- a-----c-t- --t--g---t ----t--t--
 serovar 6  ---------- ---------- a-----c--- --t--g---t ----a--t--
serovar 11  ---------- ---------- ---------- ---------- ----------
 serovar 7  ---------- ---------- ---------- ---------- ----------
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- ---------- ---------- ----------
serovar 13  ---------- ---------- ---------- ---------- ----------
 serovar 4  ---------- ---------- ---------- ---------- ----------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- ---------- ---------- ----------
 Consensus  ATGAAATTAT TAAAAAATAA GAAATTTTGA GCAATTACAC TAGGGGTAAC

            251                                                 300
 serovar 1  c-----t--- --t--aa--- -----a-a-- ---------- ----at----
 serovar 3  c-----t--- --t--aa--- -----a-a-- ---------- ----at----
serovar 14  c-----t--- --t--aa--- -----a-a-- ---------- ----at----
 serovar 6  c-----t--- --t--aa--- -----a-a-- g--------- ----at----
serovar 11  ---------- ---------- ---------- ---------- ----------
 serovar 7  ---------- ---------- ---------- ---------- ----------
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- ---------- ---------- ----------
serovar 13  ---------- ---------- ---------- ---------- ----------
 serovar 4  ---------- ---------- ---------- ---------- ----------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- ---------- ---------- ----------
 Consensus  TTTAGTGGGA GCAGGGGTAG TTGCTGTGGC AGCTTCATGT TCTAGCTCAA

            301                                                 350
 serovar 1  cc-------- ---------- -ac---t--- c--------c ------t--a
 serovar 3  c--------- ---g------ -ac---t--- c--------c -----gt--a
serovar 14  c--------- ---g------ -ac---t--- c--------c -----gt--a
 serovar 6  c--------- ---g------ --c---t--- ---------c ---t--t--a
serovar 11  ---------- ---------- ---------- ---------- ----------
 serovar 7  ---------- ---------- ---------- ---------- ----------
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- ---------- ---------- ----------
serovar 13  ---------- ---------- ---------- ---------- ----------
 serovar 4  ---------- ---------- ---------- ---------- ----------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- ---------- ---------- ----------
 Consensus  ATGTTAAATC TAAATTAAGT AGTCAACTTG TTAAATCAAA AGACGAAAAG

            351                                                 400
 serovar 1  --t-----t- -g-------- a--------c --ta-a---c ---g---t--
 serovar 3  --t-----t- -g-------- a--------c --ta-a---c ---g-a-tg-
serovar 14  --t-----t- -g-------- a--------c --ta-a---c ---g-a-tg-
 serovar 6  --t-----t- -a-------- a--------c --ta-a---c ---g---t--
serovar 11  ---------- ---------- ---------- ---------- ----------
 serovar 7  ---------- ---------- ---------- ---------- ----------
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- ---------- ---------- ---a------
serovar 13  ---------- ---------- ---------- ---------- ---a------
 serovar 4  ---------- ---------- ---------- ---------- ---a------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- ---------- ---------- ---a------
 Consensus  AGCTTTTACG CTGTTTACGA CATTGAAAAT TTCGATGATT TAACTGAAAA

            401                                                 450
 serovar 1  ---------- t-------t- -cat------ t----c---- c---------
```

```
 serovar 3  ---------- t------gta -cat------ t----c---- c---------
serovar 14  ---------- t------gta -cat------ t----c---- c---------
 serovar 6  ---------- t-------t- -cat------ t----c---- c---------
serovar 11  ---------- --------t- --a------- ---------- ----------
 serovar 7  ---------- --------t- --a------- ---------- ----------
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- ---------- ---------- ----------
serovar 13  ---------- ---------- ---------- ---------- ----------
 serovar 4  ---------- ---------- ---------- ---------- ----------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- ---------- ---------- ----------
 Consensus  TGATAAAAAA GCATTAAACG AAGCTGAATT CAATGTTGCA ATTACATCAG

            451                                                 500
 serovar 1  t------c-- ---------t ct-gtt---- -------t-- gg--ggtg--
 serovar 3  -------c-- --------gt a--cttga-- -------t-- -g--ggtg--
serovar 14  -------c-- --------gt a--cttga-- -------t-- -g--ggtg--
 serovar 6  t------c-- ---------t ct-gtt---- -------t-- gg--ggtg--
serovar 11  t--------- ---------- -----t-t-- ---------- ---------g
 serovar 7  t--------- ---------- -----t-t-- ---------- ---------g
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- ---------- ---------- ----------
serovar 13  ---------- ---------- ---------- ---------- ----------
 serovar 4  ---------- ---------- ---------- ---------- ----------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- ---------- ---------- ----------
 Consensus  CTGAAAATAA AACAGAAAAC GCAACAACAA AAGGTCACTT ACTTAACAAA

            501                                                 550
 serovar 1  -----t--c- ---------- t--------- ---c----t- ----------
 serovar 3  -----t--c- ---------- t--------- ---c----t- ----------
serovar 14  -----t--c- ---------- t--------- ---c----t- ----------
 serovar 6  -----t--c- ---------- t--------- ---c----t- ----------
serovar 11  --------c- ---------- ---------- ---------- -g--------
 serovar 7  --------c- ---------- ---------- ---------- -g--------
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- ---------- ---------- ----------
serovar 13  ---------- ---------- ---------- ---------- ----------
 serovar 4  ---------- ---------- ---------- ---------- ----------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- ---------- ---------- ----------
 Consensus  AAAATCTATG TTAAATTACC ACGTGAACCA AAAGCTAAAG AACAATTAAC

            551                                                 600
 serovar 1  ---------- ---a----ac --a-c--g-- -t--gg---g t--a------
 serovar 3  --------g- ---a----a- --a-c--g-- -t--gg---g t--a--t---
serovar 14  --------g- ---a----a- --a-c--g-- -t--gg---g t--a--t---
 serovar 6  ---------- ---a----a- --a-c--g-- -t--gg---g t--a-----a
serovar 11  ---------- --------a- ---------- -------c-- -c--------
 serovar 7  ---------- --------a- ---------- -------c-- -c--------
 serovar 9  ---------- ---------- ---------- ---------- ----------
serovar 12  ---------- ---------- ---------- ---------- ----------
serovar 13  ---------- ---------- ---------- ---------- ----------
 serovar 4  ---------- ---------- ---------- ---------- ----------
 serovar 2  ---------- ---------- ---------- ---------- ----------
 serovar 5  ---------- ---------- ---------- ---------- ----------
 serovar 8  ---------- ---------- ---------- ---------- ----------
serovar 10  ---------- ---------- ---------- ---------- ----------
 Consensus  TATTATTAAT AAAGGTGGCT TACTAAAAAC TGCATCTTTA GTATTACCTG

            601                                                 650
 serovar 1  ---------- ---------- --------ga ---------- -gctt--aa-
 serovar 3  ---------- ---------- --------ga ---------- t-c--a-cc-
serovar 14  ---------- ---------- --------ga ---------- t-c--a-cc-
```

```
 serovar 6  ---------- ---------- --------ga ---------- -gct--gaa-
serovar 11  ---------- ---------- --------a- -----a---g c-ct-atac-
 serovar 7  ---------- ---------- --------a- -----a---g c-ct-atac-
 serovar 9  -------t-- ---------- --------a- ---------a tgcg--tac-
serovar 12  ---------- ---------- --------g- -g--c----- -ca------c
serovar 13  ---------- ---------- --------g- -g--c----- -ca------c
 serovar 4  ---------- ---------- --------g- -g--c----- -ca------c
 serovar 2  -------t-- ---------- -----ta-a- gt-------- --a-------
 serovar 5  -------t-- ---------- -----ta-a- gt-------- --a-------
 serovar 8  -------t-- ---------- -----ta-a- gt-------- --a-------
serovar 10  ---------- ---------- -----ta-a- gt-------- -ca-------
 Consensus  ATAATTTGAA TTATCAAACA GAAAAAGT-G ACTTTGAAAC AA-ACCAGGA

            651                                                 700
 serovar 1  -c--aa-a-c ---...a--a ---tgtt--- g-acaac--g g--a-g---a
 serovar 3  g--aaa-a-c a-c...---- -ggtaa--a- c-acca---g g--a-g---a
serovar 14  g--aaa-a-c a-c...a-c- -gc-ggtaa- g-ac...... .........a
 serovar 6  -c-caa-a-c --g...gtaa -g-------t --a-aac--g g--a-g----
serovar 11  cca-aacc-- -gccaa-tc- --c---t-c- ccaaaa-a-g a--...--g-
 serovar 7  cca-aacc-- -gccaa-tc- --c---t-c- ccaaaa-a-g a--...--g-
 serovar 9  ccaac-cc-g a-ccta-tc- --c---tac- ccaaaa-a-g a--...--g-
serovar 12  -a----ac-- ---g-c---a ---------c --t---a--- -a-gc-c-ga
serovar 13  -a----ac-- ---g-c---a ---------c --t---a--- -a-gc-c-ga
 serovar 4  -a----ac-- ---g-c---a ---------c --t---a--- -a-gc-c-ga
 serovar 2  -------a-- -t-.....- ---------- -gt----a-- -----a----
 serovar 5  -------a-- -t-.....- ---------- -gt----a-- -----a----
 serovar 8  -------a-- -t-.....- ---------- -gt----a-- -----a----
serovar 10  ------tc-- -t-.....- -c-------- -gt---t--- ----------
 Consensus  AGTGGTG-AA CAA-C-CAGC AAAACCAGGA AA-GGTGCAA CTACACAACC

            701                                                 750
 serovar 1  -ca-c----- ----aaca-c -------ta- a--aca-caa c--ggt-a--
 serovar 3  -cc-g----- ----aaca-c c-g----ta- a--aca-c-a g--ggt-a--
serovar 14  -cc-g----- ----aaca-c ------c-gg -a-a-a-c.. ..........
 serovar 6  ---taa--aa cc--g--a-g -------ta- a--ac--ggt -a-g--c---
serovar 11  --ttgt-a-- --t-t-ga-t ttagc-atgt ---t----aa g--a------
 serovar 7  --ttgt-a-- --t-t-ga-t ttagc-atgt ---t----aa g--a------
 serovar 9  -attgt-a-- --t-t-gagt ttagc-a-gt -a-t---caa ---a------
serovar 12  -aa-c----c --t-g--c-- c-agccc-g- aa-ac--ggc -atggt---a
serovar 13  -aa-c----c --t-g--c-- c-agccc-g- aa-ac--ggc -atggt---a
 serovar 4  -aa-c----c --t-g--c-- c-agccc-g- aa-ac--ggc -atggt---a
 serovar 2  ----agt--- g--ac--c-- --------ag --gt-a---t ---a--c---
 serovar 5  ----agt--- g--ac--c-- --------ag --gt-a---t ---a--c---
 serovar 8  ----agt--- g--ac--c-- --------ag --gt-a---t ---a--c---
serovar 10  ----agt--- tc-ac--c-c --------ag --gtt----t ---c--c---
 Consensus  AGGA-CAGGT AAAG-TA-AA AACCAGGA-A TGA-GCAAC- ACA-AAACAG

            751                                                 800
 serovar 1  a-ca-caacc -gg--a---- -a-ca-c--- ......gt-- -g---a-ca-
 serovar 3  a-ca-c-a-c -gg--a---- -a-cc-g--- ......gt-- -g---a-cc-
serovar 14  a-ca-c-a-c -gg--a---- -a-ca-c--- caggtaaag- -c---a-cc-
 serovar 6  gt-a--aacc -gg--a---- -----t-a-- aacc-ggt-- -g-------t
serovar 11  ---a--tta- -tta---ttt g-ctt-gt-- t-c---ta-- -g-cga-a-t
 serovar 7  ---a--tta- -tta---ttt g-ctt-gt-- t-c---ta-- -g-cga-a-t
 serovar 9  ---a--tta- -tta---ttt g-ctc-g--- t-c---ta-- -g-cga-a-c
serovar 12  ---gcc-a-- --aac---gc aat--t---a caagc--ag- -a-------c
serovar 13  ---gcc-a-- --aac---gc aat--t---a caagc--ag- -a-------c
 serovar 4  ---gcc-a-- --aac---gc aat--t---a caagc--ag- -a-------c
 serovar 2  g--gt-gt-- --c----a-- -------gt- g-g--a-t-c -a--------
 serovar 5  g--gt-gt-- --c----a-- -------gt- g-g--a-t-c -a--------
 serovar 8  g--gt-gt-- --c----a-- -------gt- g-g--a-t-c -a--------
serovar 10  g--gt-gttc --c----c-- -------gt- g-tc-a-t-c -c--------
 Consensus  CAA-AGC-GA AA-TACAGAA CCAGGAACAG -T-AACC-AA A-AACCAGGA

            801                                                 850
 serovar 1  cca----a-g a-c------- --.......-- a--gaaca-c a-c----t--
 serovar 3  gca----a-g a-c---c-g- --.......-- a--gaaca-c --g----t--
serovar 14  gca----a-g a-c------- --c-gg-aaa ---caaca-c --g----t--
 serovar 6  --a-aacc-g gt---g---- ---t-aa-aa cc-g---a-g a-c----t--
serovar 11  c-aaaatt-t t----tt-a- ttt----aaa --t--cga-- --aa--a-gt
```

```
 serovar 7  c-aaaatt-t t----tt-a- ttt----aaa --t--cga-- --aa--a-gt
 serovar 9  c-aaaatc-t tg---tt-a- ttt----aaa --t---ga-- --aa--a-gt
serovar 12  --t----c-- ---gc-c-ga -aa-c-a--c a-tg---c-- --agccc-g-
serovar 13  --t----c-- ---gc-c-ga -aa-c-a--c a-tg---c-- --agccc-g-
 serovar 4  --t----c-- ---gc-c-ga -aa-c-a--c a-tg---c-- --agccc-g-
 serovar 2  -gt---ga-- -t-c-a---- -----g---- ----c--c-- a-c------g
 serovar 5  -gt---ga-- -t-c-a---- -----g---- ----c--c-- a-c------g
 serovar 8  -gt---ga-- -t-c-a---- -----g---- ----c--c-- a-c------g
serovar 10  -gt---tc-- -t-c------ -----g---- tc--c--c-c a-c------g
 Consensus  AA-GGTA-AA CAAAACAACC AGGAACTGGT GAAAGTA-AA CA-CAGGAAA

            851                                                900
 serovar 1  a------ca- c---..... -t-a--a-c- -ca-c----- ----a-caac
 serovar 3  a------c-- g---..... -t-a--a-c- -c--g----- ----a-caac
serovar 14  a------ca- c---caggta a-ga-ca-c- -c--g----- ----a-caac
 serovar 6  a----c--gt -a--aa---- -t-a--a-cc -ggt-a--aa cc---t-aa-
serovar 11  t--ttt--t- tt-a-tga-- at-a-tt-ag tg-g----c- g-ttt---t-
 serovar 7  t--ttt--t- tt-a-tga-- at-a-tt-ag tg-g----c- g-ttt---t-
 serovar 9  t--ctt--t- tt-a-t-a-- at-a-tt-ag tg------c- g-ttt--at-
serovar 12  aa---c--gc -at--ta--a c--gccc--- --a-c----c --t--t-caa
serovar 13  aa---c--gc -at--ta--a c--gccc--- --a-c----c --t--t-caa
 serovar 4  aa---c--gc -at--ta--a c--gccc--- --a-c----c --t--t-caa
 serovar 2  t-gtg--a-t ---aaa---- ---gt-gt-- ---t---aaa cc------t-
 serovar 5  t-gtg--a-t ---aaa---- ---gt-gt-- ---t---aaa cc------t-
 serovar 8  t-gtg--a-t ---aaa---- ---gt-gt-- ---t---aaa cc------t-
serovar 10  t-gttc-a-t ---caa---- ---gt-gttc ---t---caa cc------t-
 Consensus  -GAACAAGCA ACAGG-CCAG GAA-AG-AGA AACAACAGGT AAAGGAAG-G

            901                                                950
 serovar 1  --c--g.... ..gtaa--a- c-acaac--g g--a-g---- -c--c-a-..
 serovar 3  c-g--g.... ..gtaa--a- c-acca---g g--a-g---- -cc-g-a-..
serovar 14  --c--g-a-g t---ga-ca- c-acca---g g--a-g---- -c--c-a-ca
 serovar 6  --c--ggta- -g-------t --a-aac--g g--a-g---c -ggt-aa-aa
serovar 11  -gtt--aa-- -ggtatttat --a-t-t-t- aatt--c-tt ---c-at-t-
 serovar 7  -gtt--aa-- -ggtatttat --a-t-t-t- aatt--c-tt ---c-at-t-
 serovar 9  tgtt--at-- -gg-a-ttat --a-taa-t- aatt--cttt ---tggtaa-
serovar 12  c-agcc-a-- ---------c --t---a--- -a-gccc-g- ----c-a--c
serovar 13  c-agcc-a-- ---------c --t---a--- -a-gccc-g- ----c-a--c
 serovar 4  c-agcc-a-- ---------c --t---a--- -a-gccc-g- ----c-a--c
 serovar 2  gtga---tac ---------- -gt----a-- ---c-----c -gg--gt---
 serovar 5  gtga---tac ---------- -gt----a-- ---c-----c -gg--gt---
 serovar 8  gtga---tac ---------- -gt----a-- ---c-----c -gg--gt---
serovar 10  gtt----tac -c-------- -gt---t--- ---c-c---c -gg--gt---
 Consensus  AA-CAAC-GA AAAACCAGGA AA-GGTGCAA CTA-AAAACA AAAAAC-GGT

            951                                               1000
 serovar 1  ....---a-g ---a-ca-cc -------g-a c--c------ ......-ta-
 serovar 3  ....---a-g ---a-cc--c -------g-a c--cc-g--- ......-ta-
serovar 14  -gtaaaga-c ---a-cc--c -------g-a c--c------ c-g--aa---
 serovar 6  cc-----a-g ---c--gta- --aacc-gg- ---------- -t-aa-a-cc
serovar 11  ----t--gtt t-agt-ac-- -at----a-- ------tt-a a-gta-a-tt
 serovar 7  ----t--gtt t-agt-ac-- -at----a-- ------tt-a a-gta-a-tt
 serovar 9  ----t--gtt t-aat-ac-- -at----a-- ------tt-a a-gta-a--c
serovar 12  a-t------- c-agccc--- -aaacc-ggc --t-gta--a c---ccc---
serovar 13  a-t------- c-agccc--- -aaacc-ggc --t-gta--a c---ccc---
 serovar 4  a-t------- c-agccc--- -aaacc-ggc --t-gta--a c---ccc---
 serovar 2  ---ac----- ---c--g-ag t---g--ac- -c-a------ -------t--
 serovar 5  ---ac----- ---c--g-ag t---g--ac- -c-a------ -------t--
 serovar 8  ---ac----- ---c--g-ag t---g--ac- -c-a------ -------t--
serovar 10  tc-ac----c ---c--g-ag t---tc-ac- -c-c------ -------ttc
 Consensus  GAAGGTACAA AAC-AG-AGA AGGTAAA-AT AAAGAACCAG GAAGTGGAGA

            1001                                              1050
 serovar 1  -gaaca-c-- -----t-a-- a-ca--a-c- -ggtaa--a- c-acaac--g
 serovar 3  -gaaca-cc- g----t-a-- a-ca------ -ggtaa--a- c-acca---g
serovar 14  -caaca-cc- g----t-a-- a-ca--a-c- -gc-ggtaa- g-a.......
 serovar 6  -gg-aa-g-- -----t-a-- a--------- .......... ..........
serovar 11  ----t---c- t-tcaac--- -c-gtggt-- ---------- ..........
 serovar 7  ----t---c- t-tcaac--- -c-gtggt-- ---------- ..........
 serovar 9  tt--aa---- --t-aa---- -t-gca---a -gg------- ..........
```

```
serovar 12  --aac--ggc aat--t---a c--gc----a ---------c ------a--.
serovar 13  --aac--ggc aat--t---a c--gc----a ---------c ------a--.
 serovar 4  --aac--ggc aat--t---a c--gc----a ---------c ------a--.
 serovar 2  ----a----- -----a-gt- -tga-a-ta- ---------- -g-----a--
 serovar 5  ----a----- -----a-gt- -tga-a-ta- ---------- -g-----a--
 serovar 8  ----a----- -----a-gt- -tga-a-ta- ---------- -g-----a--
serovar 10  ----a--c-- -----a-gt- -ttc-a-ta- -c-------- -g----t---
 Consensus  AACT-CAAAA CCAGG-ACAG GAA-ACCAGC AAAACCAGGA AATGGTGCAA

            1051
 serovar 1  g-------
 serovar 3  g-------
serovar 14  ........
 serovar 6  ........
serovar 11  ........
 serovar 7  ........
 serovar 9  ........
serovar 12  ........
serovar 13  ........
 serovar 4  ........
 serovar 2  --......
 serovar 5  --......
 serovar 8  --......
serovar 10  --......
 Consensus  CTAAAGAA
```

Table 2.2. Specificity and expected lengths of ureaplasma genotyping primer pairs.

Primer pairs*	Specificity	Length of amplicons (base pairs)
UMS-125-UMA226	Ureaplasmas	403/404 for UP; 448 for UU
UMS-125-UMA222	UP	396/397
UMS-170-UMA263	UU	485
UMS3S-UMA269	UP 3	408
UMS3S-UMA314A	UP 3	576
UMS3S-UMA314A’	UP 3	578
UMS14S-UMA269	UP 14	410
UMS14S-UMA314A	UP 14	578
UMS14S-UMA314A’	UP 14	580
UMS-83-UMA1A	UP 1	582
UMS-83-UMA1A’	UP 1	584
UMS-83-UMA269’	UP 1	408
UMS-54-UMA6A	UP 6	550
UMS-54-UMA6A’	UP 6	554
UMS-54-UMA269’	UP 6	380
UMS-61-UMA2A	UU A	572
UMSUS-UMA2A	UU A	317
UMS-61-UMA10A	UU B	572
UMSUS-UMA10A	UU B	317
UMS-61-UMA4A1	UU C	545
UMSUS-UMA4A1	UU C	290
UMS-61-UMA4A2	UU C	547
UMSUS-UMA4A2	UU C	292
UMS-61-UMA9A1	UU D	553
UMSUS-UMA9A1	UU D	298
UMS-61-UMA9A2	UU D	555
UMSUS-UMA9A2	UU D	300

UMS-61-UMA7A1	UU E	338
UMS-61-UMA7A2	UU E	551
UMSUS-UMA7A2	UU E	296
UMS-61-UMA7A3	UU E	554
UMSUS-UMA7A3	UU E	299
UMS-61-UMA2A1	UU A/B	544
UMSUS-UMA2A1	UU A/B	289
UMS-61-UMA2A2	UU A/B	547
UMSUS-UMA2A2	UU A/B	292
UMS-61-UMA219	UU A–D	338
UMS-112-UMA194	UU A/D/E	357
UMS-112'-UMA194'	UU B/C	357

Notes.

* See Table 2.1. for primer sequences and genotype explainations.

2.4.4. Algorithm for serovar or genotype identification by sequencing and PCR.

A. *U. parvum* serovar identification:

A) By sequencing:

a) Sequencing UMS-125-UMA222 amplicons;

b) Using Figure 2.1. or multiple sequence aligment software (*Pileup* and *Pretty*) to identify *U. parvum* serovars.

B) By serovar-specific PCR, using primer pairs listed in Table 2.1.

B. *U. urealyticum* genotypes identification:

A) By sequencing:

a) Sequencing UMS-170-UMA263 amplicons;

b) Using Figure 2.1. or multiple sequence aligment software (*Pileup* and *Pretty*) to identify *U. urealyticum* genotypes.

B) By genotype-specific PCR, using primer pairs listed in Table 2.1. – only when necessary.

C. For most isolates or clinical specimens (suitable for most studies):

a) Sequencing UMS-125-UMA226 amplicons;

b) Using Figure 2.1. or multiple sequence aligment software (*Pileup* and *Pretty*) to identify *U. parvum* serovars or *U. urealyticum* subtypes (for 3 subtypes) (Kong *et al*., 2000).

2.5. DISCUSSION

We have described methods previously that distinguish the two ureaplasma species (Kong *et al*., 2000; chapter 1). Our previous study showed that homology between sequences of the 16S rRNA genes, 16S-23S rRNA intergenic spacer regions and urease gene subunits of serovars within each proposed species was high and these regions were not suitable for further genotyping (Kong *et al*., 2000). In this study, we sequenced the 5'-ends (including partial repetitive regions) and upstream regions of *mba* for all 14 ureaplasma serovars. Our aim was to define sequence differences

that would allow further molecular serovar or genotype identification of *U. parvum* and *U. urealyticum* (Kong *et al*., 1999a, 2000).

Our previous studies showed only three base differences between sequences of the partial 5'-ends and upstream regions of *mba* of *U. parvum* serovars 3 and 14 (Kong *et al*., 1999a, b). In this study we showed more numerous differences in nucleotide sequences immediately upstream of the repetitive regions and in the repetitive units themselves, between *U. parvum* serovars, which allowed all of them, including serovars 3 and 14, to be differentiated. Based on our previous study of partial 5'-ends and upstream regions of *mba* of *U. urealyticum* (Kong *et al*., 1999b), serovar 10 is closely related to serovars 4, 12, and 13. However, differences immediately upstream and in the repetitive units allowed serovar 10 to be separated from serovars 4/12/13. Similarly, serovar 9 was closely related to serovars 2, 5, and 8, based on sequences of the 5'-ends of *mba* (Kong *et al*., 1999b) but deletion of the repetitive region in serovar 9 allowed it to be differentiated from serovars 2/5/8. This finding is supported by the recent development of a monoclonal antibody against *U. urealyticum* serovar 9, that cross-reacts only minimally with serovar 2 (Naessens *et al*., 1998).

The present study also showed that there were 22 bases at the 5'-ends and upstream regions of *mba* of the ten serovars of *U. urealyticum*, upstream of the repetitive regions, which helped to differentiate the five *mba* genotypes. More than half of these differences were between *mba* genotype E (serovars 7/11) and the other four *mba* genotypes. Serovars 7/11 were similar to serovar 9 in that the repetitive sequences were deleted. However, their sequences differed by 14 bases at the 5'-ends of *mba* and the *mba* upstream (1-639), and 58 within sequences that corresponded with those of the repetitive regions of *mba* (640-1033), of the other serovars (Figure 2.1.). Serovars in *mba* genotypes A (serovars 2, 5 and 8), C (serovars 4, 12 and 13), and E (serovars 7 and 11) could not be differentiated further on the basis of these sequences.

Further work is required to identify other genes or other regions of the *mba* that may be used to differentiate *U. urealyticum* serovars within *mba* genotypes A, C and E. However, on the basis of our data, we suggest that genetic and antigenic differences between some serovars are so minor that further subdivision into serovars might be artificial and/or unnecessary. These data provide a better understanding of the molecular basis of serotype differentiation. Based on the sequence analysis, we designed a series of serovar- or *mba* genotype-specific primer pairs, and a practical algorithm which, after further evaluation, could be used for further study of the relationships between serovar/genotypes and diseases, if there are any.