# Molecular genotyping of human *Ureaplasma* species based on multiple-banded antigen (MBA) gene sequences

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Ureaplasma urealyticum has been divided into 14 serovars. Recently, subdivision of U. urealyticum into two species has been proposed: U. parvum (previously U. urealyticum parvo biovar), comprising four serovars (1, 3, 6, 14) and U. urealyticum (previously U. urealyticum T-960 biovar), 10 serovars (2, 4, 5, 7-13). The multiple-banded antigen (MBA) genes of these species contain both species and serovar/subtype specific sequences. Based on whole sequences of the 5'-ends of MBA genes of U. parvum serovars and partial sequences of the 5'-ends of MBA genes of U. urealyticum serovars, we previously divided each of these species into three MBA genotypes. To further elucidate the relationships between serovars, we sequenced the whole 5'-ends of MBA genes of all 10 U. urealyticum serovars and partial repetitive regions of these genes from all serovars of U. parvum and U. urealyticum. For the first time, all four serovars of U. parvum were clearly differentiated from each other. In addition, the 10 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 other regions of the MBA genes that may be used to differentiate U. urealyticum serovars within MBA genotypes A, C and E. A better understanding of the molecular basis of serotype differentiation will help to improve subtyping methods for use in studies of the pathogenesis and epidemiology of these organisms.

Keywords: Ureaplasma parvum, Ureaplasma urealyticum, MBA gene, subtyping

# INTRODUCTION

Human ureaplasmas are recognized causes of urethritis (Hewish *et al.*, 1986; Cracea *et al.*, 1985; Taylor-Robinson *et al.*, 1985), and have been associated with complications of pregnancy and prematurity (Abele-Horn *et al.*, 1997; Robertson *et al.*, 1986; Kundsin *et al.*, 1996; Hannaford *et al.*, 1999). However, as common genital tract commensals (Viarengo *et al.*, 1980; Cracea *et al.*, 1985), their pathogenic roles in individual cases are difficult to confirm (Zheng *et al.*, 1992).

Abbreviation: MBA, multiple-banded antigen.

The former species *Ureaplasma urealyticum* contained 14 serovars (Razin & Yogev, 1986; Robertson & Stemke, 1982). In the proposed new taxonomy, *Ureaplasma parvum* (previously *U. urealyticum* parvo biovar), contains four and *U. urealyticum* (previously *U. urealyticum* T-960 biovar), 10 serovars (Kong *et al.*, 1999b). The relationship between serovars and disease syndromes needs to be studied further (Grattard *et al.*, 1995; Naessens *et al.*, 1988). However, this has been limited by technical difficulties and cross-reactions associated with serotyping (Wiley & Quinn, 1984; Quinn *et al.*, 1981; Robertson & Stemke, 1979; Stemke & Robertson, 1985), even when monoclonal antibodies were used (Naessens *et al.*, 1998).

Better understanding of the genetic basis of the

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Table 1.	Oligonucleotide	primers	used ir	this study
	5			

Primer	Specificity	$T_{\mathbf{m}}$ (°C)	Sequence
UMS-125	Ureaplasma spp.	66	– 151GTA TTT GCA ATC TTT ATA TGT TTT CG-125
UMS-57	U. parvum	66	-84(A/G)(A/C)(T/C)AA ATC TTA GTG TTC ATA TTT TTT AC-57
UMSPS1*	U. parvum	68	319CCT CGT GAA CCA AAA CCT AAT G340
UMSPS2*	U. parvum	66	367GGA TTA ATC AAG ACT TCA GGT TTG390
UMA1213	UP 3/14	68	1239CTA AAG TAA TTA TTT TCC AGT AGT TTC1213
UMA1586	UP 3/14	72	1613GAT AAT CAT TCA TCT TCT CTT AAT TGT C1586
UMS3S*	UP 3	68	– 107TTA CTG TAG AAA TTA TGT AAG ATT ACC-81
UMS14S*	UP 14	68	– 109AAT TAC TGT AGA AAT TAT GTA AGA TTA AT-81
UMA269	UP 3/14	66	293AA CTA AAT GAC CTT TTT CAA GTG TAC269
UMA314A*	UP 3/14	68	463GTT GTT CTT TAC CTG GTT GTG TAG440
UMA314A'*	UP 3/14	68	<b>465</b> T <u>G/T</u> G TTG TTC TTT ACC TGG TTG TGT A <b>441</b>
UMS-54	UP 6	66	– 77AAT CTT AGT GTT CAT ATT TTT TAC TAG-54
UMA6A*	UP 6	66	464CCT GGT TCT TGA GTT TTC GGA G443
UMA6A'*	UP 6	68	468TTT ACC TGG TTC TTG AGT TTT CGG445
UMS-83	UP 1	66	– 107TTACT GTA GAA ATT ATG TAA GAT TGC-83
UMA1A*	UP 1	70	469TTT CTT TTG GTT CTT CAG TTT TTG AAG443
UMA1A'*	UP 1	68	471ATT TTC TTT TGG TTC TTC AGT TTT TGA445
UMA269′	UP 1/6	66	293ACCA AAT GAC CTT TTG TAA CTA GAT269
UMS-170	Ureaplasma spp.	66	– 195GTA TTT GCA ATC TTT ATA TGT TTT CG-170
UMS-61	U. urealyticum	66	-83TATA TTT GCA AAA CTA TAA ATA GAC AC-61
UMSUS*	U. urealyticum	66	163GTT TAC GAC ATT GAA AAT TTC GAT G187
UMAUA*	U. urealyticum	62	466GGG (G/T)(A/T)G TT(G/T) (A/C/T)AC CA(C/T) T(G/T)C CTG GTT443
UMSUS1*	U. urealyticum	62	378AAC TGC ATC T(C/T)T AG(C/T) ATT ACC T399
UMSUS2*	U. urealyticum	62	<b>397</b> CCT GAT AAT TT(G/T) AAT TAT CAA ACA G <b>421</b>
UMAUA1*	U. urealyticum	66	1537GCC CAA TTC ATA GGC TAT TAA TTG1514
UMAUA2*	U. urealyticum	64	1546AAA AAA ATA GCC CAA TTC ATA GGC1523
UMA2A1*	UU A/B	66	451TTC CTG GTT TTG TTT CAA AAC CTA T427
UMA2A2*	UU A/B	66	454CAC TTC CTG GTT TTG TTT CAA AAC431
UMA2A*	UU A	66	479CCA CTT CCT GGT TTT GTA GTT TC457
UMA10A*	UU B	68	479CCA CTT CCT GGT TGT GTA GTT GA457
UMA4A1*	UU C	68	452TT GCC TGG TTG TGT TTC GAA CTC430
UMA4A2*	UU C	68	454CAT TGC CTG GTT GTG TTT CGA AC432
UMA9A1*	UU D	66	460CTG GAG TTG GTG TAG GCG CAT440
UMA9A2*	UU D	66	462TTC TGG AGT TGG TGT AGG CGC442
UMA7A1	UU E	74	245GTA ATT GCA ACA TGG AAT TCA GTT TCA219
UMA7A2*	UU E	66	458GGT TCT GGT GTA TGA GTG CTT TT436
UMA7A3*	UU E	66	461GTT GGT TCT GGT GTA TGA GTG C440

\* Primers designed specifically for this study. All others have been previously published (see text for references). The melting temperatures  $(T_m)$  of primers were calculated by the formula:  $T_m = 4 \times \text{no. of } (G+C) + 2 \times \text{no. of } (A+T)$ .

conventional ureaplasma serotype classification will assist in development of a practical molecular serotyping system and allow further investigation of the pathogenic potential of individual subtypes/serovars (Robertson & Stemke, 1982; Kong *et al.*, 1999a). In our previous study, we sequenced three genes and adjoining regions of all 14 ureaplasma serovars and studied the phylogenetic relationships between them (Kong *et al.*, 1999b). We showed that the sequences of the 16S rRNA genes and 16S–23S rRNA intergenic spacer regions, the urease gene subunits *ureA*, *ureB*, partial *ureC* and adjoining regions – upstream of *ureA*, *ureA*–*ureB* spacer, and the *ureB*–*ureC* spacer – were generally conserved for serovars within each of the two proposed new species. Only the 5'-end regions of the MBA genes showed heterogeneity between the four serovars of *U. parvum* and the 10 serovars of *U. urealyticum*.

It has been suggested, previously, that the repetitive region of the MBA gene would contain serovar-specific sites (Watson *et al.*, 1990; Zheng *et al.*, 1996). In this study, we sequenced partial repetitive regions of the MBA genes of all 14 ureaplasma serovars, to determine whether these genes could provide further evidence to support the present serotype classification and improve our previous molecular subtyping system for *U. parvum* and *U. urealyticum* (Kong *et al.*, 1999a, 2000).

# METHODS

**Bacterial strains.** Two sets of reference strains of all 14 serovars of *U. parvum* and *U. urealyticum* were used as previously described (Kong *et al.*, 1999b). 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, AL, USA (UAB reference strains).

Oligonucleotide primers. The oligonucleotide primers used in this study are shown in Table 1. Previously published oligonucleotide primers UMS-125, UMA1213, UMA1586 (Zheng et al., 1995), UMS-57 (Kong et al., 2000), and new primers designed by us - UMSPS1, UMSPS2, UMAUA based on previously published sequences (Zheng et al., 1999; GenBank accession nos U50459, U50460, U50461) were used to sequence the repetitive regions of the MBA genes of the four U. parvum serovars. Previously published oligonucleotide primers UMS-170 (Teng et al., 1995), UMS-61 (Kong et al., 2000), and new primers designed by us – UMSUS, UMSUS1, UMSUS2, UMAUA, UMAUA1, UMAUA2 – based on the previously published sequences (Kong et al., 1999b; Zheng et al., 1999; GenBank accession nos U50459, U50460, U50461) were used to sequence the 5'end and the repetitive regions of the MBA genes of all the 10 U. urealyticum serovars.

primers - UMS3S, Additional new 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 genes of four U. parvum serovars 3, 14, 1 and 6. New primers UMA2A1, UMA2A2; UMA2A; UMA10A; UMÁ4A1, UMA4A2; UMA9A1, UMA9A2; UMA7A2 and UMA7A3, based on the sequences obtained in this study, and the previously published primer UMA7A1 (Kong et al., 2000) were designed to amplify and differentiate MBA genotypes A/B, MBA genotype A, MBA genotype B, MBA genotype C, MBA genotype D and MBA genotype E (Table 1).

**DNA preparations and PCR.** DNA preparations and PCR systems were used as previously described (Kong *et al.*, 1999a, b). To amplify the repetitive regions of the 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 genes of the 10 *U. urealyticum* serovars for sequencing. The outer primers were UMS-170/UMAUA2 and inner primers were UMS-61/UMAUA (or UMSUS/UMAUA2 (or UMSUS/UMAUA1) (Table 1).

The denaturation, annealing and elongation temperatures and times used for the first step PCR were 95 °C for 30 s, 50 °C for 30 s and 72 °C for 3 min, respectively, for 30 cycles. For the second step PCR, the denaturation, annealing and elongation temperatures and times used were 95 °C for 30 s, 55 °C for 30 s and 72 °C for 2 min, for 30 cycles. For the serovar-/MBA genotype-specific PCR, the denaturation, annealing and elongation temperatures and times used were 95 °C for 30 s, 55–62 °C (according to the  $T_{\rm m}$  value) for 30 s and 72 °C for 1 min, respectively, for 40 cycles. **Table 2.** Primer pairs used to differentiate all 14serovars of U. parvum and U. urealyticum and summaryof PCR results showing sizes of bands (amplicons)

See Table 1 for primer sequences. UP 3: *U. parvum* serovar 3; UP 14: *U. parvum* serovar 14; UP 1: *U. parvum* serovar 1; UP 6: *U. parvum* serovar 6. UU A/B: *U. urealyticum* MBA genotypes A and B, includes serovars 2, 5, 8 and 10; 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. See Figs 4 and 5 for PCR results for UMS-83 UMA269' and UMS-61 UMA7A1, respectively.

Primer pairs	Specificity (subtype/serovar)	Amplicon size (bp)
UMS3S UMA269	UP 3	400
UMS3S UMA314A	UP 3	570
UMS3S UMA314A'	UP 3	572
UMS14S UMA269	UP 14	402
UMS14S UMA314A	UP 14	572
UMS14S UMA314A'	UP 14	574
UMS-83 UMA1A	UP 1	576
UMS-83 UMA1A'	UP 1	578
UMS-83 UMA269'	UP 1	400
UMS-54 UMA6A	UP 6	544
UMS-54 UMA6A'	UP 6	548
UMS-54 UMA269'	UP 6	370
UMS-61 UMA2A1	UU A/B	539
UMSUS UMA2A1	UU A/B	289
UMS-61 UMA2A2	UU A/B	537
UMSUS UMA2A2	UU A/B	292
UMS-61 UMA2A	UU A	562
UMSUS UMA2A	UU A	317
UMS-61 UMA10A	UU B	562
UMSUS UMA10A	UU B	317
UMS-61 UMA4A1	UU C	535
UMSUS UMA4A1	UU C	290
UMS-61 UMA4A2	UU C	537
UMSUS UMA4A2	UU C	292
UMS-61 UMA9A1	UU D	543
UMSUS UMA9A1	UU D	298
UMS-61 UMA9A2	UU D	545
UMSUS UMA9A2	UU D	300
UMS-61 UMA7A1	UU E	328
UMS-61 UMA7A2	UU E	541
UMSUS UMA7A2	UU E	296
UMS-61 UMA7A3	UU E	544
UMSUS UMA7A3	UU E	299

Primer pairs used to amplify and differentiate serovars of *U. parvum* and subtypes of *U. urealyticum* are shown in Table 2. PCR products (12·5  $\mu$ l) were analysed by electrophoresis on 2·0% agarose gels, which were stained with 0·5  $\mu$ g ethidium bromide ml<sup>-1</sup>. For sequencing, PCR products of appropriate size that produced visible bands on UV illumination were further purified. For identification of individual sub-

	-200				-151
serovar 1					ag-
serovar 3					ag-
serovar 6					a
serovar 11					
serovar 9					
serovar 12					
serovar 4					
serovar 2					
serovar 8					
serovar 10	CTATTCCAA		 መምምምርርምምል እ	 እእጥጥልልልልጥ	
consensus	GIATTIGCAA	TCTTTATATG	TITICGTIAA	AATTAAAAAT	*
	-150			<i>a</i>	-101
serovar 3	-gtt-tg	-a	-c	gca	-t-tc-t-
serovar 14	-gtt-tg	-a	at	gca	-t-t-c-t-
serovar 11		-a			
serovar 7					
serovar 12				g-	
serovar 13				g-	
serovar 4 serovar 2				g-	
serovar 5					
serovar 8 serovar 10				g-	
Consensus	AAAAACAACA	TGAGATTAAA	CAAAATCTTA	ATGTTGTTAT	TATCTATACA
	-100	•	**		-51
serovar 1	-att-	g			
serovar 3 serovar 14	-att- -att-				
serovar 6	-attt-				
serovar 11 serovar 7					
serovar 9					
serovar 12					
serovar 4					
serovar 2					
serovar 8					
serovar 10 Consensus	TTCTAAAGAA	AAATATATTT	GCAAAACTAT	AAATAGACAC	AAAAAACAAT
compensas	*	*	001111101111	1221110110110110	
serovar 1	-50	tada	++-	a	-1
serovar 3	c	taga	tt-	a	a-c
serovar 14	c	taga	tt-	a	a-c
serovar 11			-g		
serovar 7			-g		
serovar 12			-g		
serovar 13			-g		
corowar /					
serovar 4 serovar 2			-g		
serovar 4 serovar 2 serovar 5 serovar 8			-g		
serovar 4 serovar 2 serovar 5 serovar 8 serovar 10			-g		
serovar 4 serovar 2 serovar 5 serovar 8 serovar 10 Consensus	AGAATAATAA	  AACTAAATTT	-g -g C <u>A</u> TATTTAGT	TTATTAGGAG	  ATCGTTATAA *
serovar 4 serovar 2 serovar 5 serovar 8 serovar 10 Consensus	AGAATAATAA 1start code	AACTAAATTT	-g -g C <u>A</u> TATTTAGT	TTATTAGGAG	ATCGTTATAA * 50
serovar 4 serovar 2 serovar 5 serovar 8 serovar 10 Consensus serovar 1 serovar 3	AGAATAATAA 1start codd	AACTAAATTT	-g -g CATATTTAGT aC aC	TTATTAGGAG	
serovar 4 serovar 2 serovar 5 serovar 8 serovar 10 Consensus serovar 1 serovar 3 serovar 14	AGAATAATAA Istart codo	AACTAAATTT 0n	-g -g CATATTTAGT ac ac-t-		
serovar 4 serovar 2 serovar 5 serovar 8 serovar 10 Consensus serovar 1 serovar 3 serovar 14 serovar 6	AGAATAATAA 1start codd	AACTAAATTT	-g -g		ATCGTTATAA * 50 
serovar 4 serovar 2 serovar 5 serovar 8 serovar 10 Consensus serovar 1 serovar 1 serovar 14 serovar 1 serovar 7	AGAATAATAA 1start codd	AACTAAATTT	-g -g CATATTTAGT a		ATCGTTATAA * 50 
serovar 4 serovar 2 serovar 5 serovar 8 serovar 10 Consensus serovar 1 serovar 3 serovar 14 serovar 6 serovar 14 serovar 7 serovar 2	AGAATAATAA Istart codd		   CATATTTAGT aC aC aC-t- aC-t- aC-t-		ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 5 serovar 8 serovar 10 Consensus serovar 11 serovar 14 serovar 14 serovar 14 serovar 7 serovar 9 serovar 13	AGAATAATAA 1start codd	AACTAAATTT on 	   CATATTTAGT aC aC-t- aC 		ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 3 serovar 8 serovar 10 Consensus serovar 1 serovar 2	AGAATAATAA Istart codd	AACTAAATTT on 	   CATATTTAGT aC aC aC 		ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 2 serovar 8 serovar 10 Consensus serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 2 serovar 4 serovar 4 serovar 5	AGAATAATAA Istart codd	AACTAAATTT AACTAAATTT Dn 			ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 2 serovar 8 serovar 10 Consensus serovar 1 serovar 1 serovar 4 serovar 1 serovar 1 serovar 2 serovar 4 serovar 4 serovar 5 serovar 10	AGAATAATAA Istart codo	AACTAAATTT n 			
serovar 2 serovar 2 serovar 3 serovar 10 Consensus serovar 10 serovar 13 serovar 14 serovar 14 serovar 15 serovar 12 serovar 13 serovar 2 serovar 2 serovar 2 serovar 8 serovar 8	AGAATAATAA Istart codd	AACTAAATTT AACTAAATTT D 	    aC aC aC aC     GAAATTTTGA	tgt tgt tgt tgt tgt tgt tgt tgt tgt tgt tgt tgt tgt tgt tgt t	
serovar 2 serovar 2 serovar 2 serovar 8 serovar 10 Consensus serovar 11 serovar 3 serovar 14 serovar 4 serovar 12 serovar 13 serovar 13 serovar 2 serovar 2 serovar 2 serovar 8	AGAATAATAA 1start codd 	AACTAAATTT 00 	-g -g		ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 2 serovar 8 serovar 10 Consensus serovar 11 serovar 1 serovar 14 serovar 14 serovar 14 serovar 11 serovar 13 serovar 13 serovar 13 serovar 2 serovar 2 serovar 8 serovar 10 Consensus serovar 1	AGAATAATAA 1start codd 	AACTAAATTT 		TTATTAGAG t-gt t-g	ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 2 serovar 8 serovar 10 Consensus serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 2 serovar 4 serovar 4 serovar 2 serovar 2 serovar 10 Consensus serovar 1 serovar 1 serovar 3 serovar 4 serovar 4 serovar 4 serovar 1 serovar 1	AGAATAATAA 1start codd 	AACTAAATTT AACTAAATTT 		tgt -tgt -tgt -tgt tgt tgt 	ATCGTTATAA * 50 
serovar 1 serovar 2 serovar 2 serovar 8 serovar 10 Consensus serovar 11 serovar 3 serovar 14 serovar 14 serovar 12 serovar 12 serovar 12 serovar 13 serovar 2 serovar 2 serovar 8 serovar 1 serovar 3 serovar 1 serovar 3 serovar 4	AGAATAATAA 1start codd 	AACTAAATTT AACTAAATTT DD 			ATCGTTATAA * 50 
serovar 1 serovar 2 serovar 2 serovar 8 serovar 10 Consensus serovar 10 serovar 13 serovar 14 serovar 14 serovar 14 serovar 15 serovar 19 serovar 10 consensus serovar 10 Consensus serovar 10 consensus serovar 10 serovar	AGAATAATAA 1start codd 	AACTAAATTT AACTAAATTT D      	-g -g		ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 5 serovar 10 Consensus serovar 10 serovar 1 serovar 3 serovar 3 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 2 serovar 1 serovar 2 serovar 10 Consensus serovar 10 consensus serovar 10 serovar 1 serovar 2 serovar 1 serovar 3 serovar 1 serovar 1 serovar 3 serovar 1 serovar 1 serovar 3 serovar 1	AGAATAATAA 1start codd 				ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 2 serovar 3 serovar 10 Consensus serovar 10 serovar 13 serovar 13 serovar 4 serovar 14 serovar 14 serovar 12 serovar 13 serovar 2 serovar 2 serovar 2 serovar 3 serovar 4 serovar 10 consensus serovar 10 serovar 10 s	AGAATAATAA 1start codd 	AACTAAATTT AACTAAATTT 			ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 2 serovar 8 serovar 10 Consensus serovar 14 serovar 3 serovar 14 serovar 6 serovar 12 serovar 12 serovar 12 serovar 2 serovar 2 serovar 2 serovar 3 serovar 4 serovar 6 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 2 serovar 2 serovar 3 serovar 1 serovar 2 serovar 2 serovar 3 serovar 4	AGAATAATAA 1start codd 	AACTAAATTT AACTAAATTT 		tgt tgt tgt tgt tgt tgt 	ATCGTTATAA * 50 
serovar 1 serovar 2 serovar 2 serovar 10 Consensus serovar 10 serovar 10 serovar 10 serovar 10 serovar 10 serovar 10 serovar 10 serovar 10 serovar 2 serovar 2 serovar 2 serovar 2 serovar 3 serovar 10 Consensus serovar 10 consensus serovar 10 serovar 10 serovar 10 serovar 2 serovar 2 serovar 2 serovar 2 serovar 2 serovar 3 serovar 10 serovar 10 serova	AGAATAATAA 1start codd 	AACTAAATTT AACTAAATTT DD      		tgt tgt tgt tgt tgt tgt 	ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 3 serovar 10 Consensus serovar 10 serovar 13 serovar 14 serovar 3 serovar 14 serovar 3 serovar 12 serovar 2 serovar 2 serovar 2 serovar 10 Consensus serovar 10 Consensus serovar 10 serovar 10 serovar 10 serovar 10 serovar 2 serovar 10 serovar 2 serovar 2 serovar 2 serovar 2 serovar 2 serovar 2 serovar 2 serovar 2 serovar 2 serovar 3	AGAATAATAA 1start codd 	-t-aa -t-aa -t-aa -t-aa	-g -g		ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 2 serovar 3 serovar 10 Consensus serovar 10 serovar 3 serovar 3 serovar 3 serovar 14 serovar 14 serovar 14 serovar 19 serovar 19 serovar 10 consensus serovar 10 consensus serovar 10 consensus	AGAATAATAA 1start codd 				ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 5 serovar 10 Consensus serovar 10 consensus serovar 11 serovar 1 serovar 14 serovar 14 serovar 1 serovar 13 serovar 2 serovar 10 consensus serovar 10 consensus serovar 10 serovar 10 serovar 10 serovar 10 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 2 serovar 1 serovar 3 serovar 4 serovar 5 serovar 1 serovar 5 serovar 1 serovar 5 serovar 1 serovar 5 serovar 1 serovar 2 serovar 1 serovar 2 serovar 1 serovar 2 serovar 1 serovar 2 serovar 1 serovar 2 serovar 3	AGAATAATAA 1start codd 	AACTAAATTT AACTAAATTT 			ATCGTTATAA * 50 
serovar 2 serovar 2 serovar 3 serovar 10 Consensus serovar 10 Serovar 10 serovar 10 serovar 10 serovar 10 serovar 10 serovar 12 serovar 12 serovar 12 serovar 12 serovar 2 serovar 2 serovar 2 serovar 2 serovar 2 serovar 10 serovar 1	AGAATAATAA 1start codd 	AACTAAATTT AACTAAATTT 		tgt tgt tgt tgt tgt 	ATCGTTATAA * 50 
serovar 1 serovar 2 serovar 3 serovar 10 Consensus serovar 10 serovar 11 serovar 13 serovar 14 serovar 14 serovar 15 serovar 12 serovar 12 serovar 2 serovar 2 serovar 2 serovar 10 Consensus serovar 10 consensus serovar 10 consensus serovar 10 serovar 10	AGAATAATAA 1start codd 	 AACTAAATTT DD      	-gt		
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serovar 6	tt-	-a	ac	ta-ac	gt
serovar 11 serovar 7					
serovar 9					
serovar 12					a
serovar 4					a
serovar 2					
serovar 8					
serovar 10					a
Consensus	AGCTTTTACG	CTGTTTTACGA	CATTGAAAAT	TTCGATGATT	TAACTGAAAA * *
	201				250
serovar 1		tt-	-cat	tc	C
serovar 14		tgta	-cat	tc	c
serovar 6		tt-	-cat	tc	c
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
00110011000		* *			
serovar 1	251 tc	t	ct-att	t	aaaata
serovar 3	c	gt	acttga	t	-gggtg
serovar 14	c	gt	acttga	t +	-gggtg
serovar 11	t		t-t		d
serovar 7	t		t-t		g
serovar 12					
serovar 13					
serovar 2					
serovar 5					
serovar 10					
Consensus	<u>C</u> TGAAAATAA *	AACAGAAAAC *	GCAAC <u>A</u> ACAA ** * **	AAGGTCACTT	ACTTAACAA <u>A</u> *
	301				350
serovar 1 serovar 3	tc-		t	ct-	
serovar 14	tc-		t	ct-	
serovar 6	tc-		t	ct-	
serovar 7	c-				-g
serovar 9 serovar 12					
serovar 13					
serovar 4 serovar 2					
serovar 5					
serovar 8 serovar 10					
serovar 8 serovar 10 Consensus	AAAATCTA <u>T</u> G	ттаааттасс	ACGTGAACCA	AAAGCTAAAG	A <u>A</u> CAATTAAC
serovar 8 serovar 10 Consensus	 AAAATCTA <u>T</u> G 351	ттаааттасс	ACGTGAACCA	AAAGCTAAAG	A <u>A</u> CAATTAAC 400
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serovar 10 Consensus serovar 1 serovar 1 serovar 14 serovar 11 serovar 7	AAAATCTATG 351 g- g- g-	TTAAATTACC	 ACGTGAACCA a-Cg a-Cg a-Cg a-Cg	ggg -tggg -tggg -tggg -tggg c-	AACAATTAAC 400 ta tat tat ta
serovar 18 serovar 10 Consensus serovar 1 serovar 14 serovar 14 serovar 11 serovar 7 serovar 12	AAAATCTATG 351 g- g- g-	a TTAAATTACC aa- aa- aa- aa- aa-	ACGTGAACCA a-Cg a-Cg a-Cg a-Cg 	ggg -tggg -tggg -tggg -tggg c	AACAATTAAC 400 ta tat taa -c
serovar 18 serovar 10 Consensus serovar 1 serovar 14 serovar 14 serovar 11 serovar 7 serovar 9 serovar 13	AAAATCTATG 351 g- g- g- 	TTAAATTACC	ACGTGAACCA	ggg -tggg -tggg -tggg -tggg -tggg -tg	AACAATTAAC 400 ta tat taa -c
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serovar 10 Consensus serovar 11 serovar 13 serovar 14 serovar 14 serovar 15 serovar 15 serovar 12 serovar 12 serovar 12 serovar 25 serovar 25 serovar 26 serovar 25 serovar 26 serovar 26 s	AAAAATCTATG 351 	ac TTAAATTACC aa- aa- a- 	a-C-g- a-C-g- a-C-g- a-C-g- a-C-g- a-C-g- a-C-g- 	-tggg -tggg -tggg -tggg -tggg -tggg -tggg c	AACAATTAAC 400 ta tat tat c
serovar 10 Consensus serovar 11 serovar 3 serovar 14 serovar 14 serovar 6 serovar 15 serovar 7 serovar 7 serovar 2 serovar 2 serovar 2 serovar 8 serovar 8	AAAATCTATG 351 		ACGTGAACCA a-Cg- a-Cg- a-cg- a-cg- ag- 		AACAATTAAC 400 t-a-t ta-t ta-t c c c c
serovar 10 Consensus serovar 1 serovar 2 serovar 2 serovar 8 serovar 1 serovar 8	AAAATCTATG 351 		ACGTGAACCA a-Cg- a-Cg- a-cg- a-cg- a-cg- 	-tggg -tggg -tggg -tggg 	AACAATTAAC 400 ta-t ta-t ta-t c c c GTATTACCTG * *
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serovar 10 Consensus serovar 11 serovar 1 serovar 1 serovar 14 serovar 14 serovar 14 serovar 13 serovar 13 serovar 13 serovar 2 serovar 2 serovar 2 serovar 3 serovar 3 serovar 3 serovar 4 serovar 4 serovar 1	AAAATCTATG 351 	TTAAATTACC	ACGTGAACCA a-C-g- a-C-g- a-C-g- a-c-g- 		AACAATTAAC 400 ta ta-t ta-t c  GIATTACCTG * * 450 -gcttaa- t-ca-cc- t-ca-cc- gctgaa- c
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serovar 10 Consensus serovar 11 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 2 serovar 1 serovar 2 serovar 2 serovar 1 serovar 3 serovar 1 serovar 2 serovar 1 serovar 2 serovar 1 serovar 3 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 2 serovar 1 serovar 1 serovar 3 serovar 1 serovar 2 serovar 1 serovar 2 serovar 1 serovar 2 serovar 2 serovar 3 serovar 3 serovar 4 serovar 2 serovar 1 serovar 2 serovar 2 serovar 3 serovar 3 serovar 4 serovar 3 serovar 4 serovar 4 serovar 4 serovar 4 serovar 5 serovar 4 serovar 4 serovar 5 serovar 4 serovar 5 serovar 4 serovar 5 serovar 4 serovar 5 serovar 4 serovar 4 serovar 4 serovar 5 serovar 4 serovar 4 serovar 5 serovar 4 serovar 4 sero	AAAATCTATG 351 	TTAAATTACC	ACGTGAACCAa-C-ga-C-ga-C-ga-C-g		AACAATTAAC 400 1a ta-t ta   
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serovar 10 Consensus serovar 10 consensus serovar 1 serovar 14 serovar 14 serovar 12 serovar 12 serovar 12 serovar 12 serovar 2 serovar 2 serovar 3 serovar 3 serovar 10 consensus serovar 11 serovar 3 serovar 10 serovar 11 serovar 3 serovar 11 serovar 4 serovar 4 serovar 2 serovar 4 serovar 3 serovar 4 serovar 3 serovar 4 serovar 4 serovar 4 serovar 5 serovar 4 serovar 10 consensus	AAAATCTATG 351 	TTAAATTACC	ACGTGAACCAa-C-ga-C-ga-C-g	AAAGCTAAAG -t-gg-g -t-gg-g -t-gg-g 	ALCAATTAAC 400 400 t-a-t t-a-t taa  
serovar 10 Consensus serovar 10 consensus serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1 serovar 3 serovar 3 serovar 1 serovar 1 serovar 3 serovar 1 serovar 1 serovar 3 serovar 1 serovar 3 serovar 1 serovar 3 serovar 4 serovar 4 serovar 2 serovar 4 serovar 2 serovar 1 serovar 3 serovar 4 serovar 3 serovar 4 serovar 1 serovar 2 serovar 1 serovar 4 serovar 4 serovar 4 serovar 4 serovar 4 serovar 4 serovar 4 serovar 5 serovar 4 serovar 5 serovar 1 serovar 4 serovar 2 serovar 4 serovar 4 sero	AAAATCTATG 351 	TTAAATTACC	ACGTGAACCAa-C-ga-C-ga-C-g		AACAATTAAC 400 400 t-a t-a-t t-a
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				701			750
serovar 12	-aa-cct-gc c-agccc-g	- aa-acggc -atggta	serovar 1	cggtaaa-	- c-acaacg	ga-g	-cc-a
serovar 13	-aa-cct-gc c-agccc-g	- aa-acggc -atggta	serovar 3	c-gggtaaa-	- c-accag	ga-g	-cc-g-a
serovar 4	-aa-cct-gc c-agccc-g	- aa-acggc -atggta	serovar 14	cg-a-g tga-ca	- c-accag	ga-g	-cc-a-ca
serovar 2	agt gacca	ıggt-atac	serovar 6	cggtagt	:a-aacg	ga-gc	-ggt-aa-aa
serovar 5	agt gacca	uggt-atac	serovar 11	-gttaaggtatttat	:a-t-t-t-	aattc-tt	c-at-t-
serovar 8	agt gacca	ıggt-atac	serovar 7	-gttaaggtatttat	:a-t-t-t-	aattc-tt	c-at-t-
serovar 10	agt tc-acc-ca	uggtttcc	serovar 9	tgttatgg-a-ttat	:a-taa-t-	aattcttt	tggtaa-
Consensus	AGGA-CAGGT AAAG-TA-AA AACCAGGA-	A TGA-GCAAC- ACA-AAACAG	serovar 12	c-agcc-a	ta	-a-gccc-g-	c-ac
	551	600	serovar 13	c-agcc-a	ta	-a-gccc-g-	c-ac
serovar 1	a-ca-caacc -ggaa-ca-c	gtga-ca-	serovar 4	c-agcc-a	cta	-a-gccc-g-	c-ac
serovar 3	a-ca-c-a-c -ggaa-cc-g	gtga-cc-	serovar 2	gtgatac	gta	c	-gggt
serovar 14	a-ca-c-a-c -ggaa-ca-c	- caggtaaagca-cc-	serovar 5	gtgatac	gta	cc	-gggt
serovar 6	gt-aaacc -ggat-a-	- aacc-ggtgt	serovar 8	gtgatac	gta	c	-gggt
serovar 11	attattattt g-ctt-gt-	- t-ctag-cga-a-t	serovar 10		g		-gggt
serovar 7	attattattt g-ctt-gt-	- t-ctag-cga-a-t	Consensus	AA-CAAC-GA AAAACCAGGA	A AA-GGIGCAA	CIM-MAMACA	AAAAAAC-GGI
serovar 9	attattattt g-ctc-g	- t-ctag-cga-a-c		751			800
serovar 12	gcc-aaacgc aatt	a caagcagac	serovar 1	a-ga-ca-co	cg-a	cc	ta-
serovar 13	gcc-aaacgc aatt	a caagcagac	serovar 3	a-ga-cc0	:g-a	ccc-g	ta-
serovar 4	gcc-aaacgc aatt	a caagcagac	serovar 14	-gtaaaga-ca-ccd	cg-a	cc	c-gaa
serovar 2	ggt-gtcagt	:- g-ga-t-c -a	serovar 6	cca-gcgta	aacc-gg-		-t-aa-a-cc
serovar 5	ggt-gtcagt	- g-ga-t-c -a	serovar 11	tgtt t-agt-ac	ata	tt-a	a-gta-a-tt
serovar 8	ggt-gtcagt	:- g-ga-t-c -a	serovar 7	tgtt t-agt-ac	ata	tt-a	a-gta-a-tt
serovar 10	ggt-gttcccgt	:- g-tc-a-t-c -c	serovar 9	tgtt t-aat-ac	ata	tt-a	a-gta-ac
Consensus	CAA-AGC-GA AA-TACAGAA CCAGGAACA	AG -T-AACC-AA A-AACCAGGA	serovar 12	a-t c-agccc	aaacc-ggc	t-gtaa	cccc
	601	650	serovar 13	a-t c-agccc	aaacc-ggc	t-gtaa	cccc
serovar 1	CCaa-g a-C	- agaaca-c a-ct	serovar 4	a-t c-agccc	aaacc-ggc	t-gtaa	cccc
serovar 3	gcaa-g a-cc-g	- agaaca-cgt	serovar 2	accg-ag	g tgac-	-c-a	t
serovar 14	gcaa-g a-cc-gg-aa	acaaca-cgt	serovar 5	accg-ag	g tgac-	-c-a	t
serovar 6	a-aacc-g gtgt-aa-a	a cc-ga-g a-ct	serovar 8	accg-ag	g tgac-	-c-a	(
serovar 11	c-aaaatt-t ttt-a- tttaa	atcgaaaa-gt	serovar 10		A ACCUMANA AM	-C-C	CAACTOCACA
serovar 7	c-aaaatt-t ttt-a- tttaa	atcgaaaa-gt	consensus	GAAGGIACAA AAC-AG-AGA	A AGGIAAA-AI	AAAGAACCAG	GAAGIGGAGA
serovar 9	c-aaaatc-t tgtt-a- tttaa	atgaaaa-gt		801			850
serovar 12	tcqc-c-ga -aa-c-a	c a-tgcagccc-g-	serovar 1	-gaaca-ct-a	- a-caa-c-	-ggtaaa-	c-acaacg
serovar 13	tcgc-c-ga -aa-c-a	c a-tgcagccc-g-	serovar 3	-gaaca-cc- gt-a	- a-ca	-ggtaaa-	c-accag
serovar 4	tcgc-c-ga -aa-c-a	c a-tgcagccc-g-	serovar 14	-caaca-cc- gt-a	- a-caa-c-	-gc-ggtaa-	g-a
serovar 2	-gtgat-c-ag	cc a-cg	serovar 6	-gg-aa-gt-a	- a		
serovar 5	-gtgat-c-ag	cc a-cg	serovar 11	tc- t-tcaac	c-gtggt		• • • • • • • • • • •
serovar 8	-gtgat-c-ag	cc a-cg	serovar /	tc- t-tcaac	c-gtggt		
serovar 10	-gttct-cg	tccc a-cg	serovar 9	ttaat-aa	t-gcaa	-gg	
Consensus	AA-GGTA-AA CAAAACAACC AGGAACTGO	ST GAAAGTA-AA CA-CAGGAAA	serovar 12	aacggc aatta	a cgca	C	a
	651	700	serovar 13	aacggc aatta	a cgca	0	a
serovar 1	aca- ct-aa-	ca-ca-caac	serovar 4	aacyyc aatta	a cgca	C	a
serovar 3	ac gt-aa-g	cga-caac	Serovar Z	aa-gt	tga-a-ta-		-ga
serovar 14	aca- ccaggta a-ga-ca-	ccga-caac	Serovar S	aa-gt	tga-a-ta-		-ga
serovar 6	acgt -aaat-aa-g	c -ggt-aaa cct-aa-	serovar 10	a ge	ttc-a-ta-		-at
serovar 11	ttttt- tt-a-tga at-a-tt-a	ag tg-gc- g-tttt-	Consensus	AACT-CAAAA CCAGG-ACA	GAN-ACCAGC	AAAACCAGGA	AATGGTGCAA
serovar 7	ttttt- tt-a-tga at-a-tt-a	ag tg-gc- g-tttt-	conscisus				
serovar 9	tcttt- tt-a-t-a at-a-tt-a	ag tgc- g-tttat-		851			
serovar 12	aacgc -attaa cgccc	a-cctt-caa	serovar 1	g			
serovar 13	aacgc -attaa cgccc	a-cctt-caa	serovar 3	g			
serovar 4	aacgc -attaa cgccc	a-cctt-caa	serovar 14				
serovar 2	t-gtga-taaagt-gt-	taaa cct-	serovar b				
serovar 5	t-gtga-taaagt-gt-	taaa cct-	serovar 11				
serovar 8	t-gtga-taaagt-gt-	taaa cct-	serovar /				
serovar 10	t-gttc-a-tcaagt-gtf	ctcaa cct-	Serovar 10				
Consensus	-GAACAAGCA ACAGG-CCAG GAA-AG-AG	A AACAACAGGT AAAGGAAG-G	Serovar 12				
			serovar 13	• • • • • • • • •			
			Serovar 2				
			serovar 5				
			serovar 8				
			serovar 10				
			Consensus	CTAAAGAA			

**Fig. 1.** Multiple sequence alignment of the MBA gene DNA sequences of 14 serovars of *U. parvum* and *U. urealyticum* (ATCC strains). \*45 sites of nucleotide differences between *U. parvum* serovars; underlining indicates 22 sites of nucleotide differences between *U. urealyticum* serovars.

types, the presence of PCR amplicons of expected length on ultraviolet transillumination were accepted as positive.

Sequencing and phylogenetic analysis. The PCR products of the 5'-end and the repetitive regions of the MBA genes of all the 14 ureaplasma serovars were sequenced using Applied Biosystems (ABI) *Taq* DyeDexoy terminator cyclesequencing kits according to standard protocols. Primers UMSPS1 (or UMSPS2) were used as sequencing primers 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).

The amino acid sequences were derived by converting nucleotide sequences using Translate program from the Readseq program groups provided in WebANGIS, ANGIS (Australian National Genomic Information Service), 3rd version (mycoplasma translation codes were used). 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. Phylogenetic relationships based on the MBA gene DNA sequences for the 14 serovars of *U. parvum* and *U. urealy-ticum* (ATCC strains) were studied using CLUSTAL for alignment and PHYLIP to construct the phylogenetic tree. The tree was formed using *Chlamydia trachomatis* (GenBank accession no. AE001315) as outgroup and was bootstrapped with 100 replications.

Nucleotide sequence accession numbers. The sequence data used in the paper are in GenBank/EMBL/DDBJ with the following accession numbers: AF056982, AF056983, AF056984; AF055358, AF055359, AF055360, AF055361, AF055362, AF055363, AF055364, AF055365, AF055366, AF055367 (Kong *et al.*, 1999a, b).

# RESULTS

#### 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. urealy*- *ticum*, inner primers UMS-61/UMAUA amplified portions of all 10 serovars whereas UMSUS/UMAUA2 (or UMSUS/UMAUA1) amplified portions of seven (all except serovars 9, 7 and 11).

#### Comparative study of the nucleotide sequences and amino acid sequences of the 5'-end region and partial repetitive regions of MBA genes

There were base differences at 45 (45/601 = 7.5%) sites at the 5'-end of MBA genes (-200-450) among the four serovars of *U. parvum* (Kong *et al.*, 1999b) (Fig. 1). There were amino acids differences at 19 (19/150 = 12.6%) sites at the N-terminus of MBA (1-159) among the four serovars of *U. parvum* (Fig. 2). There were base differences at 22 (22/634 = 3.5%) sites at the 5'-end of MBA genes (-200-439) among the 10 serovars of *U. urealyticum* (Fig. 1) and amino acid differences at 9 (9/146 = 6.2%) sites at the N-terminus of MBA (1-146) (Fig. 2).

Nucleotide and amino acid sequences of the MBA gene repetitive units of U. parvum and U. urealyticum are shown in Table 3. They begin in the vicinity of nucleotide 451 (Fig. 1) and amino acid 151 (Fig. 2). There were differences between sequences from all 4 U. parvum serovars. Sequences from serovars 2, 5 and 8 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 2/8 amino acids; this serovar 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 (Table 3). However, there were differences between serovar 9 and serovars 7/11 in 58 (58/391 = 14.8%) nucleotide bases in the region 440-833 and 14 (14/634 = 2.2%) in the region -200-439 at the 5'-end of MBA genes (Fig. 1). There were corresponding differences between serovar 9 and serovars 7/11 in 31 amino acids (31/130 = 23.8%) in the regions of 151–283 and 6 (6/146 = 4.1%) in the region 1-146 at the N-terminus of MBA (Fig. 2). These differences defined two additional MBA genotypes, D (serovar 9) and E (serovars 7 and 11).

# The phylogenetic trees of the 14 serovars of *U. parvum* and *U. urealyticum*

Phylogenetic tree, based on the nucleotide sequences of the 5'-ends and partial repetitive regions of MBA genes is shown in Fig. 3. Serovars 3 and 14 of *U. parvum* are most closely related, with serovars 1 and 6 more distant. The 10 serovars of *U. urealyticum* form five MBA genotypes as outlined above. MBA genotypes A, C and E are separate clusters of three, three and two serovars, respectively, with the MBA genotype B (a single serovar) located between MBA genotypes A and C; MBA genotype D (also a single serovar) is located between MBA genotypes C and E (Fig. 3).

	1				5.0
serovar 1	1	-m		-n-t	nat
serovar 3		-m		-n-t	nat-g-
serovar 14	1	-m		-n-t	nat-g-
serovar 6		-m		-n-t	t
serovar 7					
serovar 9					
serovar 12					
serovar 13					
serovar 2					
serovar 5					
serovar 8					
serovar 10	MULLENNEREN	A TOTI CUOTI VC	ACUMUNUA NCC	CCONTROLLO	COLUNCADER
consensus	MUTTINUKLAM	ATTEGVIEVG	AGVVAVAASC	SSSIIVERES	* * * *
	51				100
serovar 1		-ks	sia-	1v	lvvge
serovar 3		-ksnd	s-snia-	1s	tlevge
serovar 14		-ksnd	s-snia-	1s	tlevge
serovar 11			t	v	i
serovar 7			t	v	i
serovar 9					
serovar 12		n			
serovar 4		n			
serovar 2					
serovar 5					
serovar 8					
Serovar 10	SEVAUVDIEN	n	ALNEAFENVA	TTCAENETEN	ATTRCUT I NK
consensus	SFIAVIBLEN	**	**	* *	***
	101	~~~		14	150
serovar 1		-pns	-sisg-	lis	n
serovar 14		-pns	-sisg-	lis	n
serovar 6		-pn	-sisg-	li-n	n
serovar 11				a	d-k
serovar /				a	d-k
serovar 12					a
serovar 13					e
serovar 4					e
serovar 2					-n-gk
serovar 8					-n-gk
serovar 10					-n-g
Consensus	KIYVKLPREP	KAKEQLTIIN	KGGLLKTASL	VLPDNLNYQT	EKV_FETQPG
		· ·		**	
	151	Ŷ		**	200
serovar 1	151 askt	ee-kenv-eq	p-k-qqp-ke	qqkeqqpg	200 keqqpgkeqq
serovar 1 serovar 3	151 askt tg	ee-kenv-eq keqpagkeqp	p-k-qqp-ke a-k-qpa-ke	qqkeqqpg qpa-keqpag	200 keqqpgkeqq keqpagkeqp
serovar 1 serovar 3 serovar 14	151 tq tq	ee-kenv-eq keqpagkeqp keqqp	p-k-qqp-ke a-k-qpa-ke a-k-q	qqkeqqpg qpa-keqpag qpa-keq	200 keqqpgkeqq keqpagkeqp qpagkeqq
serovar 1 serovar 3 serovar 14 serovar 1	151 tq tq apkt	ee-kenv-eq keqpagkeqp keqqp qe-gkep-ke	p-k-qqp-ke a-k-qpa-ke a-k-q p-kkepg	** qqkeqqpg qpa-keqpag qpa-keq kekepgke dakakta-v-	200 keqqpgkeqq keqpagkeqp qpagkeqq kepgke
serovar 1 serovar 3 serovar 14 serovar 6 serovar 11 serovar 7	151 tq tq apkt -th-pept-t -th-pept-t	ee-kenv-eq keqpagkeqp keqqp qe-gkep-ke pt-apdka pt-apdka	p-k-qqp-ke a-k-qpa-ke a-k-q p-kkepg vvsnvefs-v vvsnvefs-v	** qqkeqqpg qpa-keqpag qpa-keq kekepgke dakakta-v- dakakta-v-	200 keqqpgkeqq keqpagkeqp qpagkeqq kepgke ltfalvvqlk ltfalvvqlk
serovar 1 serovar 3 serovar 14 serovar 6 serovar 11 serovar 7 serovar 9	151 tq tq apkt -th-pept-t -th-pept-t nap-ptpe-t	ee-kenv-eq keqpagkeqp keqqp qe-gkep-ke pt-ap-dka pt-ap-dka ptp-dka	p-k-qqp-ke a-k-qpa-ke a-k-q p-kkepg vvsnvefs-v vvsnvefs-v ivsnvefs-v	<pre>** qqkeqqpg qpa-keqpag qpa-keq kekepgke dakakta-v- dakakta-v- naqtkta-v-</pre>	200 keqqpgkeqq keqpagkeqp qpagkeqq kepgke ltfalvvqlk ltfalvvqlk ltfa-avqlk
serovar 1 serovar 3 serovar 14 serovar 6 serovar 11 serovar 7 serovar 9 serovar 12	151 askt tq apkt -th-pept-t nap-ptpe-t n-t-s-e	ee-kenv-eq keqpagkeqp keqqp qe-gkep-ke pt-apdka pt-apdka n-t-sp-kp-	p-k-qqp-ke a-k-qpa-ke a-k-q p-kkepg vvsnvefs-v vvsnvefs-v ntspekpg	qqkeqqpg qpa-keqpag qpa-keqa kekepgke dakakta-v- dakakta-v- naqtkta-v- ngttsppg	200 keqqpgkeqq keqpagkeqp qpagkeqq kepgke ltfalvvqlk ltfalvvqlk ltfa-avqlk n-tt-pe
serovar 1 serovar 3 serovar 14 serovar 6 serovar 11 serovar 7 serovar 12 serovar 13	151 askt tq apkt -th-pept-t nap-ptpe-t n-t-s-e n-t-s-e	ee-kenv-eq keqpagkeqp keqqp qe-gkep-ke pt-apdka pt-apdka ptpdka n-t-sp-kp- n-t-sp-kp-	p-k-qqp-ke a-k-qpa-ke a-k-q p-kkepg vvsnvefs-v vvsnvefs-v ivsnvefs-v n-tspekpg n-tspekpg	qqkeqqpg qpa-keqpag qpa-keqpag kekepgke dakakta-v- dakakta-v- naqtkta-v- ngttsppg ngttsppg	200 keqqpgkeqq keqpagkeqp qpagkeqq kepgke ltfalvvqlk ltfalvvqlk ltfa-avqlk n-tt-pe n-tt-pe
serovar 1 serovar 14 serovar 6 serovar 7 serovar 7 serovar 9 serovar 12 serovar 13 serovar 13	151 tq apkt -th-pept-t n-ts-e n-t-s-e n-t-s-e e	ee-kenv-eq keqpagkeqp keqqp qe-gkep-ke pt-ap-dka pt-ap-dka nt-sp-kp- n-t-sp-kp- n-t-sp-kp- n-t-sp-kp-	p-k-qqp-ke a-k-qpa-ke a-k-q p-kkepg vvsnvefs-v ivsnvefs-v ivsnvefs-v ivsnvefs-v ntspekpg ntspekpg ntspekpg et-k	qqkeqqpg qpa-keqpag qpa-keq kekepgke dakakta-v- dakakta-v- naqtkta-v- ngttsppg ngttsppg ngttsppg tksq-tt-	200 keqqpgkeqp qpagkeqp qpagkeq tfalvvqlk ltfalvvqlk ltfalvvqlk n-tt-pe n-tt-pe n-tt-pe
serovar 1 serovar 3 serovar 14 serovar 6 serovar 11 serovar 7 serovar 9 serovar 12 serovar 13 serovar 4 serovar 5	151 tq apkt -th-pept-t nap-ptpe-t n-t-s-e n-t-s-e n-e	ee-kenv-eq keqpagkeqp keqqp qe-gkep-ke pt-ap-dka pt-ap-dka ptp-dka n-t-sp-kp- n-t-sp-kp- n-t-sp-kp- ep-s-	p-k-qqp-ke a-k-qpa-ke a-k-q p-kkepg vvsnvefs-v vvsnvefs-v vvsnvefs-v ntspekpg ntspekpg et-kt	qqkeqqpg qpa-keqpag dpa-keq kekepske dakakta-v- dakakta-v- ngttsppg ngttsppg tksg-tt- tksg-tt-	200 keqqpgkeqq keqpagkeqp kepgke ltfalvvqlk ltfalvvqlk ltfa-avqlk n-tt-pe n-tt-pe ett
serovar 1 serovar 3 serovar 14 serovar 6 serovar 11 serovar 7 serovar 12 serovar 12 serovar 4 serovar 2 serovar 2 serovar 8	151 askt tq apkt -th-pept-t ndp-ptpet nap-ptpet n-t-s-e n-t-s-e e	ee-kenv-eq keqpagkeqp keqqp ge-gkep-ke pt-ap-dka pt-ap-dka ptp-dka n-t-sp-kp- n-t-sp-kp- n-t-sp-kp- -ep-s- -ep-s-	p-k-qqp-ke a-k-qpa-ke a-k-qpa-ke p-kkepg vvsnvefs-v vvsnvefs-v ivsnvefs-v ntspekpg ntspekpg et-kt et-kt	qqkeqqpg qpa-keqpag qpa-keq kekepgke dakakta-v- dakakta-v- ngttsppg ngttsppg ngttsppg tksg-tt- tksg-tt-	200 keqqpgkeqq keqpagkeqp kepgke ltfalvvqlk ltfalvvqlk ltfa-avqlk n-tt-pe n-tt-pe ett ett
serovar 1 serovar 14 serovar 14 serovar 15 serovar 11 serovar 12 serovar 12 serovar 13 serovar 13 serovar 2 serovar 2 serovar 2 serovar 8 serovar 10	151 askt askt -th-pept-t nt-s-e n-t-s-e e e	ee-kenv-eq keqpagkeqp qe-gkep-ke pt-apdka pt-apdka n-t-sp-kp- n-t-sp-kp- n-t-sp-kp- n-ep-s- -ep-s- -ep-s- -ep-s- -ep-s-	p-k-qqp-ke a-k-qpa-ke a-k-qpa-ke p-kkepg vvsnvefs-v ivsnvefs-v et-tspekpg tet-kt tet-kt	qqkeqqpg qpa-keqpag qpa-keqi kekepske dakakta-v- naqtkta-v- ngttsppg ngttsp-pg ngttsp-pg ngttsp-sg- ttksg-tt- tksg-tt- tksg-tt- tqsgattq	200 keqDgkeqg keqDagkeqp qpagkeqn tfalvvqlk ltfalvvqlk ltfalvvqlk n-tt-pe n-tt-pe n-tt-pe ett ett ett
serovar 1 serovar 14 serovar 14 serovar 16 serovar 11 serovar 9 serovar 22 serovar 22 serovar 4 serovar 4 serovar 5 serovar 10 Consensus	151 akt tq akt .th-pept-t .th-pept-t n-t-s-e n-t-s-e -e -e sG-TTP-KPG SG-TTP-KPG	ee-kenv-eq keqpagkeqp keqqp pt-ap-dka pt-ap-dka n-t-sp-kp- n-t-sp-kp- n-t-sp-kp- n-ep-s- -ep-s- -sqp-s- SGPTTKEG-G	p-k-qqp-ke a-k-qpa-ke p-kkepg vvsnvefs-v vvsnvefs-v ivsnvefs-v ivsnvefs-v ivsnvefs-v et-kt et-kt et-kt et-kt et-kt	qqkeqqpg qpa-keqpag qpa-keq kekepske dakakta-v- naqtkta-v- naqtktsp-pg ngttsp-pg ngttsp-pg ngttsp-pg stksg-tt- tksg-tt- tksg-tt- tksg-tt- tksg-tt- tksg-tt- tksg-tt- tgsgsttq PGEK-K	200 keqgpgkeqg qpagkeqp kepgke- ltfalvvqlk ltfa-avqlk n-tt-pe n-tt-pe n-tt-pe ett ett PGSGSKPG
serovar 1 serovar 3 serovar 14 serovar 1 serovar 7 serovar 1 serovar 2 serovar 2 serovar 4 serovar 2 serovar 8 serovar 10 Consensus	151 askt tg apkt -th-pept-t nap-ptpe-t n-t-s-e n-t-s-e -e -sg- SG-TTP-KPG **** 201	ee-kenv-eq keqpagkeqp ge-gkep-ke pt-apdka pt-apdka nt-sp-kp- n-t-sp-kp- n-t-sp-kp- -eep-s- -eep-s- -sqp-s- SGPTTKEG-G	p-k-qqp-ke a-k-qa-ke p-kkepg vvsnvefs-v ivsnvefs-v ivsnvefs-v ivsnvefs-n ivsnvefs-v et-spekpg et-kt et-kt et-kt et-kt et-kt et-kt	qqkeqqpg qpa-keqpag qpa-keqpag dakakta-v- naqtkta-v- ngttsp-pg ngttsp-pg tk-sg-tt- tk-sg-tt- tk-sg-tt- tk-sg-tt- tq-sgsttq PGEK-K	200 keqgpgkeqg qpagkeqg kepgke ltfalvvqlk ltfalvvqlk ltfalvvqlk ltfa-avqlk n-tt-pe n-tt-pe ett ett PGGSSKPG 250
serovar 1 serovar 14 serovar 14 serovar 16 serovar 11 serovar 11 serovar 12 serovar 12 serovar 2 serovar 2 serovar 2 serovar 3 serovar 4 serovar 10 Consensus	151 akt tq- akt .th-pept-t .th-pept-t nt-s-e n-t-s-e .e SG-TTP-KPG *** 201 peqgp-	ee-kenv-eq keqpagkecpw pt-ap-dka pt-ap-dka pt-p-dka n-t-sp-kp- n-t-sp-kp- n-t-sp-kp- sgPTTKEG-G keqqke	p-k-qqp-ke a-k-qqp-ke a-k-q p-kkepg vvsnvefs-v vvsnvefs-v ivsnvefs-v ntspekpg ntspekpg et-kt et-kt st-qst -GTEPGSGE- qqk-qq-	qqkeqqpg qpa-keqpag qpa-keqpag dakakta-v- dakakta-v- naqtkta-v- ngttsp-pg ngttsp-pg ngttsp-pg ngttsp-sg- tksg-tt- tk-sg-tt-tk- tk-sg-tt-tk- tk-sg-tt-tk- tk-sg-tt-tk- tk-sg-tt-tk- tk-sg-tt-tk- tk- tk-sg-tt-tk- tk- tk- tk-sg-tt-tk- tk- tk- tk-sg-tt-tk- tk- tk-sg-tt-tk- tk- tk- tk- tk- tk- tk- tk- tk-	200 keqqpgkeqq qpagkeqq kepgke-q ltfalvvqlk ltfalvvqlk ltfalvvqlk ltfa-avglk n-tt-pe n-tt-pe n-tt-pe ett PGSGSKPG 250 qqpskeqq
serovar 1 serovar 14 serovar 14 serovar 16 serovar 11 serovar 12 serovar 12 serovar 2 serovar 2 serovar 4 serovar 3 serovar 10 Consensus serovar 1	151 akt tq akt .th-pept-t nt-s-e n-t-s-e n-t-s-e -e -sq SG-TTP-KPG 201 peqqp- ***	ee-kenv-eq keqpagkeqp pt-ap-dka pt-ap-dka pt-pdka pt-pdka nt-sp-kp- -ep-s- -ep-s- -sqp-s- SGPTTKEG-G keqqke	p-k-qqp-ke a-k-qa-ke wsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v et-kt et-kt et-kt -GTEPGSGE- gg-k-gg gpa-k-gga- ma-k-ga-	qqkeqqpg qpa-keqpag qpa-keqs kekepgke dakakta-v- dakakta-v- naqtkta-v- naqtkta-v- naqtkta-y- pgttsp-pg tk-sg-tt- tk-sg-tt- tk-sg-tt- tk-sg-tt- tk-sg-tt- tk-sg-tt- tk-sg-tt- k-sg-tk- k-sg-tk- k-sg-gtke keqqpgke keq-agke	200 keqpgkeqq qpagkeqq kepgke-q tfalvvqlk ltfalvvqlk ltfa-avqlk n-tt-pe n-tt-pe n-tt-pe ett PGSGSKPG 250 qpgkeqq qpag.keqp
serovar 1 serovar 3 serovar 14 serovar 6 serovar 9 serovar 9 serovar 9 serovar 12 serovar 2 serovar 2 serovar 4 serovar 2 serovar 3 serovar 1 serovar 1 serovar 1 serovar 1 serovar 1	151 askt tg apkt -th-pept-t n-t-s-e n-t-s-e -e SG-TTP-KPG *** 201 peqpp- pagkeqpa- aeqpa- pagkeqpa-	ee-kenv-eq keqpagkeqp keqqp qe-gkep-ke pt-apdka pt-apdka ptpdka n-t-sp-kp- n-t-sp-kp- n-t-sp-kp- n-t-sp-kp- sGPTTKEG-G keqqkeqagg keqq-ag-qg keq-ag-ag	p-k-qqp-ke a-k-qa-ke a-k-q p-kkepg vvsnvefs-v ivsnvefs-v ivsnvefs-v ivsnvefs-v ivsnvefs-v ivsnvefs-v ivsnvefs-v t-tspekpg et-kt et-kt et-kt et-kt gq-k-qq qpa-k-qpa- qpa-k-qpa- qpa-k-qpa-	qqkeqqpg qpa-keqpag qpa-keqpag dakakta-v- naqtkta-v- naqtkta-v- ngttsp-pg ngttsp-pg tk-sg-tt- tk-sg-tt- tk-sg-tt- tk-sg-tt- tk-sg-tk- x-g-EK-K keqagke gkeqq-agke	200 keqqpgkeqq qpagkeqq kepgke-q ltfalvvqlk ltfalvvqlk ltfalvvqlk ltfarv
serovar 1 serovar 14 serovar 14 serovar 16 serovar 11 serovar 11 serovar 12 serovar 12 serovar 2 serovar 2 serovar 2 serovar 3 serovar 10 Consensus serovar 1 serovar 14 serovar 14	151 askt tg- apkt th-pept-t n-t-s-e n-t-s-e re	ee-kenv-eq keqpagkeqp gt-ap-dka pt-ap-dka ptp-dka n-t-sp-kp- n-t-sp-kp- n-t-sp-kp- s-ep-s- -ep-s- s-gPTTKEG-G keqqke keqqaakeg pgkkeqg l-k-set-v	p-k-qqp-ke a-k-qq p-kkepg vvsnwefs-v vvsnwefs-v vvsnwefs-v ntspekpg ntspekpg et-kt et-kt et-kt et-kt qq-k-qq qpa-k-qqa- qpa-k-qq-a kek-pgke ket,pgke	qqkeqqpg qpa-keqpag qpa-keqpag daakata-v- dakakta-v- dakakta-v- ngttsppg ngttsppg ngttsppg tksg-tt- tksg-tt- tksg-tt- tksg-tt- tksg-tt- tksg-tt- tksg-tt- .keqqpgke .keq-agke kepgke- .cat-lse	200 keqqpgkeqq qpagkeqq kepgke- ltfalvvqlk ltfalvvqlk ltfa-avqlk n-tt-pe n-tt-pe n-tt-pe ett PGSGSKPG qpgg.keqq qpag.keqq heggkep-ke kegikep-ke
serovar 1 serovar 14 serovar 14 serovar 16 serovar 11 serovar 9 serovar 12 serovar 2 serovar 2 serovar 4 serovar 3 serovar 3 serovar 10 Consensus serovar 11 serovar 14 serovar 14 serovar 7	151 askt tq apkt t-th-pept-t nt-s-e n-t-s-e -e -sq- SG-TTP-KPG Peqgp- aeqgp- askeqga- kepg-e-gke de-q-11-1t	ee-kenv-eq keqpagkeqp pt-ap-dka pt-ap-dka pt-p-dka pt-p-dka nt-sp-kp- n-t-sp-kp- n-t-sp-kp- -ep-s- -ep-s- -s-qp-s- SGPTTKEG-G keqqke keqqagkeq pgkkepg 1-k-set-v	p-k-qqp-ke a-k-qa-ke wsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v vvsavefa-v et-kt et-kt et-kt et-kt et-kt et-kt gq-k-qq qpa-k-qqa- ke-k-pgke dlvll.	qqkeqqpg qpa-keqpag qpa-keqs. ke-kepgke dakakta-v- dakakta-v- nagtkta-v- ngttsp-pg ngttsp-pg tk-sg-tt- tk-sg-tt- tk-sg-tt- tk-sg-tt- tk-sg-tt- tk-sg-tt- k-sg-tt-k-sg-tt- k-sg-tt-k-sg-t	200 keqpgkeqq qpagkeqq kepgke-q ltfalvvqlk ltfalvvqlk ltfa-avqlk n-tt-pe n-tt-pe ett PGSGSKPG 250 qqpakeqq qpagkeqq kepgkep-ke lk-giykv-k
serovar 1 serovar 14 serovar 14 serovar 16 serovar 11 serovar 9 serovar 12 serovar 2 serovar 2 serovar 2 serovar 2 serovar 3 serovar 3 serovar 14 serovar 1 serovar 1 serovar 1 serovar 2 serovar 2 serovar 3	151 askt askt .th-pept-t .th-pept-t n-t-s-e n-t-s-e e e SG-TTP-FXPG 201 Pedga- pagkedgaa- edga- pagkedga de-q-11-1t de-q-11-1t de-q-11-1t	ee-kenv-eq keqpagkecpb gt-ap-dka pt-ap-dka pt-p-dka n-t-sp-kp- n-t-sp-kp- n-t-sp-kp- n-t-sp-kp- sc-p-s- sc-p-s- sc-p-s- sc-p-s- sc-p-s- sc-rkeb kecqake kecqake kecqake kecqa_s-cps l-k-set-v l-k-set-v l-k-set-v	p-k-qqp-ke a-k-qqp-ke a-k-qpa-ke wsnvefs-v vvsnvefs-v vvsnvefs-v n-tspekpg n-tspekpg n-tspekpg et-kt et-kt st-qst st-qst gqpa-k-qpa- qpa-k-qpa- qpa-k-qpa- ke-k-ppa- ke-k-ppa- ke-k-qpa- ke-k-qpa- dul1 dulq-1	qqkeqqpg qpa-keqpag qpa-keqs kekepgke dakakta-v- dakakta-v- naqtkta-v- ngttsppg ngttsppg ngttsppg ngttsppg tksg-tt- tksg-tt- tk-sg-tt- tksg-tt-tk- tksg-tt-tk- tksg-tt-tk- tksg-tt-tksg-tt-tk- tk-sg-tt-tk- tksg-tt-tk- tk-sg-tt-tk- tksg-tt-tk- tksg	200 keqqpgkeqq qpagkeqq kepgke- Ltfalvvqlk Ltfalvvqlk Ltfa-avqlk n-tt-pe n-tt-pe ett PGSGSKPG QCD4.keqq QCD42.keqq QCD42.eqq QCD42.eqq Lx-giykv-k lk-giykv-k lk-giykv-k lk-giykv-k
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**Fig. 2.** Multiple sequence alignment of the MBA amino acid sequences of 14 serovars of *U. parvum* and *U. urealyticum* (ATCC strains). \*19 sites of amino acid differences between *U. parvum* serovars; underlining indicates nine sites of amino acid differences between *U. urealyticum* serovars.

# The specificity of U. urealyticum subtyping primers

All the ATCC and UAB reference strains of *U. parvum* and *U. urealyticum* were correctly identified by the

# **Table 3.** Nucleotide and amino acid sequences of repetitive units of the MBA genes of 14 serovars of *U. parvum* and *U. urealyticum*

UP: *U. parvum*; UU: *U. urealyticum*. UU MBA genotype A: includes serovars 2, 5 and 8; UU MBA genotype B: includes serovar 10; UU MBA genotype C: includes serovars 4, 12 and 13; UU MBA genotype D: includes serovar 9; UU MBA genotype E: includes serovars 7 and 11.

Serovar/subtype	Nucleotide sequence	Amino acid sequence
UP Serovar 1	CAA CAA CCA GGT AAA GAA	QQPGKE
UP Serovar 3	CAA CCA GCA GGT AAA GAA	QPAGKE
UP Serovar 6	GGT AAA GAA CCA	PGKE
UP Serovar 14	CAA CAA CCA GCA GGT AAA GAA	Q QPAGKE
UU MBA genotype A	ACA AAA CCA GGA AGT GGT GAA ACT	TKPGSGET
UU MBA genotype B	ACA CAA CCA GGA AGT GGT TCA ACT	TQPGSGST
UU MBA genotype C	ACA AGC CCA GAA AAA CCA GGC AAT GGT ACA	TSPEKPGNGT
UU MBA genotype D	_	—
UU MBA genotype E	_	



**Fig. 3.** Phylogenetic tree for 14 serovars of *U. parvum* (UP) and *U. urealyticum* (UU) (ATCC strains) based on the MBA gene DNA sequences. CLUSTAL was used for alignment, and PHYLIP was used for constructing the phylogenetic tree. The tree was formed using *Chlamydia trachomatis* (GenBank no. AE001315) as outgroup and was bootstrapped with 100 replications.

serovar-/MBA genotype-specific primers. The results of PCR for all the serovars of *U. parvum* and *U. urealyticum*, using the 33 subtype-specific primer pairs to amplify the 5'-end of the MBA genes are summarized in Table 2 and representative examples are shown as Figs 4 and 5.

Our primary evaluation showed that the serovars and MBA genotypes (corresponding with serovars) of *U. parvum* and *U. urealyticum* were identified, specifically, using primer pairs as shown in Table 2.

# DISCUSSION

Various phenotypic and molecular methods have been described previously to distinguish the two main groups of human ureaplasmas (formerly two biovars of *U. urealyticum*, now proposed species *U. parvum* 

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 M



**Fig. 4.** Results of PCR amplification of the 5'-end of MBA genes of all 14 serovars of *U. parvum* and *U. urealyticum* using primers UMS-83 and UMA269'. *U. parvum* consists of serovars 1, 3, 6 and 14; *U. urealyticum* consists of serovars 2, 4, 5, 7, 8, 9, 10, 11, 12 and 13. Lanes: M, molecular mass markers  $\phi$ X174 DNA/Hinfl; 1 and 16, *U. parvum* serovar 1 ATCC strain and UAB reference strain; 2–5, *U. urealyticum* serovar 2, *U. parvum* serovar 3, *U. urealyticum* serovars 4 and 5 ATCC strains; 8–15, *U. urealyticum* serovar 14 ATCC strains.

and U. urealyticum) (previously summarized by Kong et al., 1999b). 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 could not be used for further subtyping (Kong et al., 1999b, 2000). However, sequence differences between the partial 5'-end regions of the MBA genes allowed each species to be divided into three genotypes (Kong et al., 2000). It had been suggested previously that the repetitive region of the MBA gene should also contain serovar-specific definition sites (Zheng et al., 1996). Therefore, in this study, we sequenced the whole 5'-end regions of the MBA genes of the 10 serovars of U. urealvticum and partial repetitive regions of the MBA genes for all 14 ureaplasma serovars. Our aim was to define sequence differences that would allow further molecular identi-



**Fig. 5.** Results of PCR amplification of the 5'-end of MBA genes of all 14 serovars of *U. parvum* and *U. urealyticum* using primers UMS-61 and UMA7A1. *U. parvum* consists of serovars 1, 3, 6 and 14; *U. urealyticum* consists of serovars 2, 4, 5, 7, 8, 9, 10, 11, 12 and 13. Lanes: M, molecular mass markers  $\phi$ X174 DNA/Hinf1; 1 and 8, *U. urealyticum* serovar 7 UAB reference strain and ATCC strain; 12 and 16, *U. urealyticum* serovar 11 ATCC strain and UAB reference strain; 2–7, *U. parvum* serovar 1, *U. urealyticum* serovar 3, *U. urealyticum* serovars 4, 5 and *U. parvum* serovar 6 ATCC strains; 9–11, *U. urealyticum* serovars 12, 13 and *U. parvum* serovar 14 ATCC strains.

fication of serovars or additional MBA genotypes of *U. parvum* and *U. urealyticum* (Kong *et al.*, 2000).

Our previous studies showed only three base differences between sequences of the 5'-end of MBA gene of U. parvum serovars 3 and 14 (Kong et al., 1999a, b). In this study we showed more numerous differences in nucleotide and amino acid sequences of the repetitive units, 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'end sequences of the MBA genes of U. urealyticum (Kong et al., 1999b), serovar 10 is closely related to serovars 4, 12 and 13. However, differences in nucleotide and amino acid sequences immediately upstream of the repetitive regions and in the repetitive units themselves, 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'-end of the MBA genes (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 minimally only with serovar 2 (Naessens *et al.*, 1998a).

The present study also showed that there were 22 bases at the 5'-end of the MBA genes of the 10 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'-end of MBA genes and 58 within sequences that corresponded with those of the repetitive regions of MBA genes of the other serovars (Fig. 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.

Reliable differentiation between the serovars of U. urealyticum using phenotypic methods is difficult. Antigenic cross-reactions between serovars 2 and 5 (Wiley & Quinn, 1984; Quinn et al., 1981), 4 and 8 (Quinn et al., 1981), and 8, 2 and 4 (Robertson & Stemke, 1979) have been described. Because of variable strain selection from frequently mixed cultures, the reproducibility of serovar determination between primary and secondary plating of isolates was only 83 %; it increased only to 87% on multiple, secondary cultures (Stemke & Roberston, 1985). The fact that genetic differences between some serovars are minor has been confirmed by demonstration that a single amino acid difference between the MBA of serovars 3 and 14 of U. parvum accounts for epitope differences that can be distinguished using type-specific monoclonal anibodies (Zheng et al., 1996). Arbitrarily primed PCR, using a pairwise combination of primers, was able to differentiate only a few of the 10 serovars of U. urealyticum (Grattard et al., 1995).

Further work is required to identify other genes or other regions of the MBA genes 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 phylogenetic analysis, we designed a series of serovar-/MBA genotype-specific primer pairs to subtype each *Ureaplasma* species and are developing a more practical subtyping system that can be used for further study of the relationship between subtypes and diseases.

# ACKNOWLEDGEMENTS

We thank Mark Wheeler for assistance with sequencing.

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