Proposal of *Ureaplasma parvum* sp. nov. and emended description of *Ureaplasma urealyticum* (Shepard et al. 1974) Robertson et al. 2001

Janet A. Robertson,¹ Gerald W. Stemke,² John W. Davis, Jr,³ Ryō Harasawa,⁴ David Thirkell,⁵ Fanrong Kong,⁶ Maurice C. Shepard⁷† and Denys K. Ford⁸‡

Author for correspondence: Janet A. Robertson. Tel: +1 780 492 2335. Fax: +1 780 492 7521.
e-mail: janet.robertson@ualberta.ca

The phenotypic and genotypic properties of *Ureaplasma urealyticum* (family *Mycoplasmataceae*, order *Mycoplasmatales*, class *Mollicutes*) are reviewed here. The 14 recognized serovar standard strains found in humans exhibit no serological cross-reactivity with ureaplasmas from other hosts and uniquely express human immunooglibulin A1 protease activity. However, they exhibit many characteristics which place them in two distinct clusters known as the parvo biovar (or biovar 1 or B) and the T960T biovar (or biovar 2 or A). Established phenotypic markers of the biovars include clustering of antigenic types, polypeptide patterns of whole-cell preparations, differential inhibition by manganese, and polymorphism among their ureases, pyrophosphatases and diaphorases. Established genotypic markers of the biovars are DNA–DNA hybridization of 60% between biovars, and distinctive RFLP patterns and genome sizes. Divergent nucleotide sequences of several highly conserved genes attest to the phylogenetic distinctiveness of the two biovars. PCRs founded upon the sequences for 16S rRNA, the 16S–23S rRNA intergenic regions, the genus-defining urease, the serovar-defining, multiple-banded antigen genes or randomly amplified polymorphic DNA tests differentiate the biovars unambiguously. With the availability of rapid, reliable and economical tests for biovar determination, it is now appropriate to propose that the taxonomic status of *U. urealyticum* be emended. Serovar standard strains exhibiting traits of biovar parvo (serovars 1, 3, 6 and 14) will be designated as a separate species, *Ureaplasma parvum* sp. nov., as befits its smaller genome size. The serovar 3 standard (strain 27T) will be the type strain of *U. parvum* and is represented by ATCC 27815T and NCTC 11736T. Serovar standard strains exhibiting traits of biovar T960T (2, 4, 5, 7, 8T, 9, 10, 11, 12 and 13) will retain the *U. urealyticum* designation and type strain, the serovar 8 standard (strain T960T'), represented by ATCC 27618T and NCTC 10177T.

Keywords: *Ureaplasma parvum*, *Ureaplasma urealyticum*, biovars, phenotyping, genotyping
INTRODUCTION

Organisms of the class Mollicutes are exceptionally small prokaryotic cells which lack a cell wall. The Mycoplasmataceae, Family I of the order Mycoplasmatales, are non-helical, sterol-requiring (and thus digitonin-sensitive) strains which are not obligate anaerobes. They comprise two genera: Mycoplasma and Ureaplasma. The taxonomy of the genus Ureaplasma was last described by Taylor-Robinson & Gourlay (1984). Phylogenetically, on the basis of 16S rRNA gene homology, the genus Ureaplasma falls within the Mycoplasma pneumoniae clade of the Mollicutes (Weisburg et al., 1989). Strains of Ureaplasma from humans are distinct from Ureaplasma strains from other mammalian and avian hosts and comprise two clusters (Fig. 1). One cluster or biovar is composed of strains of an emended Ureaplasma urealyticum species and the other of the proposed species Ureaplasma parvum (Robertson et al., 1994; Harasawa et al., 1996; Stemke & Robertson, 1996).

The tiny (‘T’) strain mycoplasmas were discovered in human urogenital tract samples by Shepard (1954, 1956). Their status as self-replicating biological entities was confirmed by Ford et al. (1962). The most notable features of these isolates were their tiny (‘T’) colonies, which were significantly smaller than those of other members of the Mycoplasmataceae, themselves generally undetectable by the naked eye, and the unusual pH growth optimum of 6.0–6.5 (Shepard & Lunceford, 1965). Unique among the Mollicutes, the ‘T’ strains were shown to hydrolyse urea (Purcell et al., 1966; Shepard, 1966; Ford & McDonald, 1967; Shepard & Lunceford, 1967) but not glucose (Black, 1973), other carbohydrates (Robertson & Howard, 1988) or arginine (Woodson et al., 1965). Urease activity was shown to be associated with, and, later, essential to the generation of ATP by a chemiosmotic mechanism (Romano et al., 1980; Smith et al., 1993). The genus and the first species, U. urealyticum, were named by Shepard et al. (1974).

Although ureaplasmas from humans are serologically distinct from members of the genus isolated subsequently from other mammals or avian species, they also are the only isolates which have been shown to produce human immunoglobulin A1 protease activity (Robertson et al., 1984; Kilian et al., 1984; Stemke et al., 1984; Kapatais-Zoumbos et al., 1985).

Ureaplasma colonies usually develop after 1–3 d incubation. Depending upon the strain and cultural conditions, ureaplasmas sometimes produce tiny ‘fried-egg’ colonies but more often produce only part of such a typical Mollicutes growth pattern (Fig. 2a). Although ureaplasma colony sizes can be enhanced by incorporating a suitable buffer into the medium (Manchee & Taylor-Robinson, 1969), they infrequently reach diameters similar to those of the so-called ‘large colony mycoplasmas’ from which they can be differentiated by positive tests for urease activity (Fig. 2b). Cultures in broth can reach populations of approximately 10^8 organisms ml^-1, but, because of the small cellular mass, they do not cause detectable turbidity (Shepard et al. 1974).

The spherical or cocccobacillary-shaped cells typical of the genus Ureaplasma have been reported to have diameters of between 0.1 and 1.0 µm. Extremely small or large cells or filamentous cells may result from adverse cultural conditions or as artefacts of preparation for microscopy (Shepard & Masover, 1979). By morphometry, using electron microscopy, cellular diameters of exponential phase cells were determined

![Fig. 1. Phylogenetic tree, prepared using the DNAMAN program, based upon 16S rRNA gene sequences and bootstrapped with 300 replications. It indicates the relationships between the type strains of the emended species Ureaplasma urealyticum strain T960T (ATCC 27618T = NCTC 10177T) and sp. nov. Ureaplasma parvum strain 27T (ATCC 27815T = NCTC 11736T) proposed herein and the type species of the other named species of the genus Ureaplasma. The latter are as follows: the avian isolate Ureaplasma gallerorae D6-1T (ATCC 43346T = NCTC 11707T) (Koshimizu et al., 1987); the feline isolates Ureaplasma cati FT2-B1T (ATCC 49226T = NCTC 11710T) and Ureaplasma felinum F2T (ATCC 49229T = NCTC 11709T) (Harasawa et al., 1990); the canine isolate Ureaplasma canigenitalium D6P-C1T (ATCC 51252T) (Harasawa et al., 1993); and the bovine isolate Ureaplasma diversum A417T (ATCC 43321T = NCTC 10182T) (Howard & Gourlay, 1982). The most recently deposited GenBank DNA sequences for Ureaplasma were used; these were, respectively, AF073450, AF073456, U62937, D78649, D78651, D78648 and D78650 (Harasawa et al., 1996; Stemke & Robertson, 1996; Kong et al., 1999). The rRNA gene sequence included as a basis for comparison was that of Mycoplasma pneumoniae of the same Mollicutes clade, as represented by the type strain FH (ATCC 15531T = NCTC 10119T) (Somerson et al., 1963) (GenBank accession no. M29061; Weisburg et al., 1989), not the sequence of strain M129 (GenBank accession no. U00089; Himmlerich et al., 1996).]
to be ~0.5 μm (Robertson et al., 1983). Typical ultrastructural features are shown in Fig. 2(c). Although members of *Mollicutes* are of Gram-positive lineage, in the absence of a bacterial cell wall they cannot hold the crystal violet of the Gram stain but take on the colour of the counter-stain. The minute cells are, however, rarely discernable in Gram-stained preparations examined at ×1000 magnification.

Under the early conventions which were applied to the taxonomy of other members of *Mollicutes*, each strain with a distinct antigenic specificity was accorded separate species status. However, when updating the minimal standards for the taxonomy of the class *Mollicutes*, the International Committee on Systematic Bacteriology’s Subcommittee on the Taxonomy of *Mollicutes* (ICSB/STM, 1979) echoed the opinion of Shepard et al. (1974) in stating that ‘The utility of the growth inhibition and immunofluorescence serological reactions [serotyping tests] as criteria for species distinction, does not apply within the genus *Ureaplasma* and is of untested validity in other genera’. The criteria to be applied to the taxonomy of *Ureaplasma* species were to include serology, PAGE and DNA–DNA hybridization (ICSB/STM, 1984, 1995). Although serological tests differentiated ureaplasmas from various hosts, intraspecies serological heterogeneity was the first means used to distinguish individual isolates within the species *U. urealyticum* (Ford, 1967; Black, 1970). Fourteen distinct specificities have been identified (Robertson & Stemke, 1982). However, because the serotyping methods were demanding to perform and the multiple serovars of clinical isolates confounded interpretation, this approach was not recommended when large numbers of strains were involved (Stemke & Robertson, 1985).

The phenotypic and genotypic evidence that has accumulated over the last two decades has confirmed fundamental differences among human ureaplasmas which were consistent with two species. The recent, independent confirmation of several species-defining criteria, the development of several practical tests for unambiguously differentiating them, as well as the considerable interest in their roles in human disease, make this taxonomic adjustment timely.

**METHODS**

**Strains.** The sources and designations of the serovar standard strains of the two biovars of the ureaplasmas associated with humans are provided in Table 1, along with culture-collection accession numbers. Serological identification of the serovar standard strains has been made by using metabolic inhibition tests (Purcell et al., 1966; Robertson & Stemke, 1979), the mycoplasmacidal test (Lin et al., 1972) or an indirect colony epifluorescence test (Stemke & Robertson, 1981).

**Media.** The serovar standard strains were cultivated at 35–37 °C in media supplemented with animal serum as source of sterols (Edward, 1947) but modified by the addition of exogenous urea and various other growth factors. Incubation was at 35–37 °C. Cultures in liquid medium were usually incubated in air, whilst those on solid medium were usually incubated under conditions of reduced oxygen.
Table 1. Sources and identification of the serovar standard strains of the two biovars of the present species *Ureaplasma urealyticum*

Strains shown in bold are the representative strains for each biovar or species.

<table>
<thead>
<tr>
<th>Name*</th>
<th>Serovar no. †</th>
<th>Culture collection number(s) ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biovar described as 1, B or Parvo§ and proposed as <em>Ureaplasma parvum</em> sp. nov.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27†</td>
<td>3 (III)</td>
<td>ATCC 27815†; NCTC 11736†</td>
</tr>
<tr>
<td>7</td>
<td>1 (I)</td>
<td>ATCC 27813</td>
</tr>
<tr>
<td>Pi</td>
<td>6 (VI)</td>
<td>ATCC 27818</td>
</tr>
<tr>
<td>U26</td>
<td>14</td>
<td>ATCC 33697</td>
</tr>
<tr>
<td>Biovar described as 2, A or T960†§ proposed as the <em>Ureaplasma urealyticum</em> emend. T960†-(CX8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>2 (II)</td>
<td>ATCC 27814</td>
</tr>
<tr>
<td>58</td>
<td>4 (IV)</td>
<td>ATCC 27816</td>
</tr>
<tr>
<td>354</td>
<td>5 (V)</td>
<td>ATCC 27817</td>
</tr>
<tr>
<td>Co</td>
<td>7 (VII)</td>
<td>ATCC 27819</td>
</tr>
<tr>
<td>Vancouver</td>
<td>9</td>
<td>ATCC 33175</td>
</tr>
<tr>
<td>Western</td>
<td>10</td>
<td>ATCC 33699</td>
</tr>
<tr>
<td>K2</td>
<td>11</td>
<td>ATCC 33695</td>
</tr>
<tr>
<td>U24</td>
<td>12</td>
<td>ATCC 33696</td>
</tr>
<tr>
<td>U38</td>
<td>13</td>
<td>ATCC 33698</td>
</tr>
</tbody>
</table>

* The sources of the strains are listed in Robertson & Stemke (1982).
† Although Black (1970) had introduced Roman numeral designations (shown here in parentheses) for the first eight antigenic specificities, when expanding the serotyping scheme Robertson & Stemke (1982) introduced Arabic numerals to reduce the possibility of labelling errors.
‡ ATCC, American Type Culture Collection, Manassas, VA, USA; NCTC, National Culture Type Collection, Colindale, UK.
§ The clusters of strains having similar traits were first referred to as biotypes 1 and 2 (Robertson, 1978). Effect of manganese on the growth of *Ureaplasma urealyticum* (T-strain mycoplasma). In Abstracts of the American Society for Microbiology, p. 74) and were later referred to as Group B and Group A, respectively (Mouches et al., 1981). To avoid confusion with the numbered serovars, Robertson et al. (1990) introduced the biovar descriptors T-960 and *parvo* (Latin for small); the latter referred to the smaller genome size of the members of the *parvo* biovar. The biovars of all serotype standard strains have been confirmed by PCRs based upon differences in the 16S rRNA sequences (Robertson et al., 1993); for confirmation of biovar or a newly defined species, see Table 4.

RESULTS AND DISCUSSION

Initial characterization

Initial characterization of the first set of (eight) serovars of the initial *U. urealyticum* included over 30 criteria (Black, 1973). Apart from already recognized antigenic variation (Ford, 1967; Table 1) and the ability of the serovar 3 standard strain to haemadsorb (Manchee & Taylor-Robinson, 1969), these strains appeared homogeneous. The first genomic studies, showing a G+C composition of between 27 and 28 mol% (Bak & Black, 1968; Black et al., 1972; Howard et al., 1978) and genome sizes between 4·1 and 4·8 × 10⁶ Da (701 and 735 kpb) (Bak et al., 1969; Black et al., 1972), reinforced this view. The DNA of the recently sequenced genome of strain 27† (serovar 3) had a G+C content of 26 mol% (Glass et al., 2000). Newer methodology has resulted in revised genome sizes (see Genome size, below).

Determination of phenotypic traits. References to the detailed, study-specific methods used for the preparation of cells, PAGE, immunoblotting, and determination of the effect of Mn³⁺ on growth are provided in Table 2.

Determination of genotypic traits. References to the detailed, study-specific methods used for preparation of cells, DNA isolation, hybridization, endonuclease digestion, agarose gel electrophoresis, nucleotide sequencing, PCR, and other molecular biological techniques are provided in Table 3.

Strain clustering to biovar

Upon further investigation into non-serologic phenotypy (Table 2) and genotypy (Table 3), the heterogeneity of *U. urealyticum* was revealed. However, by then, ureaplasmas had been found in hosts other than humans. Concern was centred not upon the taxonomic
### Table 2. Phenotypic traits that distinguish the two biovars of ureaplasmas from humans

<table>
<thead>
<tr>
<th>Trait</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoretic patterns of cell proteins</td>
<td>Sayed &amp; Kenny (1980)</td>
</tr>
<tr>
<td>Isoelectric focusing and SDS-PAGE</td>
<td>Howard et al. (1981)</td>
</tr>
<tr>
<td>One-dimensional SDS-PAGE</td>
<td>Mouches et al. (1981)</td>
</tr>
<tr>
<td>Two-dimensional SDS-PAGE</td>
<td>Swenson et al. (1983)</td>
</tr>
<tr>
<td>Differential growth response to 1 mM (\text{Mn}^{2+})*</td>
<td>Robertson &amp; Chen (1984)</td>
</tr>
<tr>
<td>Immunoblots of cell proteins</td>
<td>Horowitz et al. (1986)</td>
</tr>
<tr>
<td>Polypeptides recognized by polyclonal antisera</td>
<td>Lee &amp; Kenny (1987)</td>
</tr>
<tr>
<td>Membrane proteins recognized by polyclonal antisera</td>
<td>Thirkell et al. (1989)</td>
</tr>
<tr>
<td>72 kDa urease subunit recognized by monoclonal antibodies</td>
<td>Thirkell et al. (1990)</td>
</tr>
<tr>
<td>Enzyme polymorphism</td>
<td>Davis et al. (1987); Davis &amp; Villaneuva (1990)</td>
</tr>
</tbody>
</table>

* Growth of the parvo and T960T biovars was inhibited temporarily and permanently, respectively. Serovar 13 gave an anomalous response (Stemler et al., 1987).

† Zymogram available in IJSEM Online (http://ijs.sgmjournals.org).

### Table 3. Genotypic traits that distinguish the two biovars of ureaplasmas from humans

<table>
<thead>
<tr>
<th>Trait</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA–DNA hybridization (%)</td>
<td>Christiansen et al. (1981); Harasawa et al. (1991)</td>
</tr>
<tr>
<td>RFLP patterns of DNA</td>
<td>Razin et al. (1983), Cocks &amp; Finch (1987)</td>
</tr>
<tr>
<td>Genomic DNA</td>
<td>Harasawa et al. (1991)</td>
</tr>
<tr>
<td>Genomic DNA probed with rRNA genes</td>
<td>Teng et al. (1994)</td>
</tr>
<tr>
<td>5' Regions of multiple-banded antigen genes</td>
<td>Neyrolles et al. (1996)*</td>
</tr>
<tr>
<td>Urease genes</td>
<td>Robertson et al. (1990)</td>
</tr>
<tr>
<td>Genome size determined by PFGE</td>
<td></td>
</tr>
<tr>
<td>Gene heterogeneity</td>
<td>Blanchard (1990)*, Neyrolles et al. (1996), Kong et al. (1999)</td>
</tr>
<tr>
<td>Urease gene subunits and adjoining upstream regions</td>
<td>Robertson et al. (1993, 1994), Kong et al. (1999)</td>
</tr>
<tr>
<td>16S rRNA genes</td>
<td>Harasawa &amp; Kanamoto (1999), Kong et al. (1999)</td>
</tr>
<tr>
<td>16S–23S rRNA intergenic spacer regions</td>
<td>Kong et al. (1999)</td>
</tr>
<tr>
<td>5' Regions of multiple-banded antigen genes</td>
<td>Kong et al. (1999)</td>
</tr>
<tr>
<td>Biovar-specific PCR amplification</td>
<td></td>
</tr>
<tr>
<td>With primers based upon 16S rRNA</td>
<td>Robertson et al. (1993)</td>
</tr>
<tr>
<td>With arbitrarily primed PCRs</td>
<td>Grattard et al. (1995a)</td>
</tr>
</tbody>
</table>

* Although Blanchard (1990) identified the first biovar-specific PCR, two misidentified strains resulted in an anomalous pattern of partition for the serovar standard strains, and its usefulness was not immediately recognized. The correction was reported by Teng et al. (1994) and independently confirmed by Kong et al. (1999).

Status of strains from the same (human) host species but on whether isolates from another host (bovine) warranted separate species status (e.g. Howard & Gourlay, 1982). Until now, of the six named Ureaplasma species (Fig. 1), only the serologically distinguishable Ureaplasma cati and Ureaplasma felinum (Harasawa et al., 1990) are associated with the same host (the cat). Most interestingly, when the serovar standard strains of ureaplasmas from humans showed differing traits, usually they were partitioned to the same two clusters or biovars (Table 1). Only with hindsight was it clearly recognized that the intra-species, serological cross-reactions in ureaplasmas from humans were biovar-restricted (e.g. Robertson et al., 1993).

### Exceptional traits

Traits which do not partition the strains to one or other biovar are few. For example, the ability of colonies of the serovar 3 standard strain to adsorb erythrocytes (Manchee & Taylor-Robinson, 1969)
pertained to that strain but not to all isolates carrying the serovar 3 determinant or classified within the parvo biovar (Robertson & Sherburne, 1991). The distribution of certain other phenotypic traits among the serovar standards, e.g. extramembranous layers (Robertson & Smook, 1976), phospholipase A₁, A₂, and C activities (De Silva & Quinn, 1986, 1999), or antimicrobial-susceptibility patterns has not been established.

**Role in disease**

The ureaplasmas are mucosal parasites which, in humans, may be found as genito-urinary commensals but also as agents of urethritis; moreover, in immunocompromised patients they may cause abscesses and pyogenic arthritis. Although generally considered opportunistic pathogens, the extent of ureaplasmal pathogenicity is poorly understood (Taylor-Robinson, 1996). Indeed, it is the hope of clinical microbiologists that taxonomic recognition of these two distinct species will serve to clarify their role in human infections. In view of such uncertainty, pathogenicity as a phenotypic trait was excluded from this assessment.

**DNA–DNA hybridization**

Although we have adopted a polyphasic approach, genomic traits have been particularly helpful in resolving the taxonomic status of ureaplasmas of humans. In the last edition of *Bergey’s Manual*, Johnson (1984) indicated that, although taxonomic decisions were indeed arbitrary, experience had shown that strains showing > 70% DNA homology (intragroup) invariably were the same species, whilst those showing < 60% homology (intergroup) were not. Two decades ago, in 1980, the first DNA–DNA hybridization studies, using a direct binding method with membrane filters, showed parvo biovar strain hybridizations of 90–102% within that cluster but 38–60% with strains of the T960T biovar. The data were 69–100% and 49–52%, respectively, for the T960T biovar. Only one of the 36 hybridizations was in the 60–70% transition zone (Christiansen et al., 1981). Later, when the hybridization patterns were confirmed using a hydroxyapatite column method (Harasawa et al., 1991) and respecting the maximum 5°C ΔTm requirement (Wayne et al., 1987), none of the 14 serovars showed homologies within the transition zone. Thus, the results of both studies were consistent with *U. urealyticum* comprising two distinct taxa. In the determination of bacterial species, the superiority of DNA–DNA hybridization data over more recently available 16S rRNA gene sequences has yet to be successfully challenged. Stackebrandt & Goebel (1994) have allowed that the more conveniently determined 16S rRNA gene sequences may be substituted for hybridization determinations unless heterogeneity is ≤ 3% when their resolution is limited. They have emphasized, however, that ‘What the threshold value of 70% [DNA–DNA homology] does not take into account is the possibility that the tempo and mode of changes differ in different prokaryotic microbes.’ Thus, the concept of the relatively fast evolution of the *Mollicutes* proposed by Maniloff (1992) would merely enhance the already convincing hybridization data.

**Genome sizes**

In 1990, a decade after the first DNA–DNA hybridization studies had shown that ureaplasmas of humans fell into two biovars, genome size (as determined by then new PFGE methodology) revealed a second genomic criterion that supported separate species status. Unlike the previously reported estimates (701–735 kbp), based upon buoyant-density determinations (Black et al., 1972), PFGE data showed a broader range of genome sizes (760–1140 kbp). Furthermore, there was a discontinuity of ~70 kbp between genomes of the parvo biovar (769 kbp) and the T960T biovar (840–1140 kbp) (Robertson et al., 1990) which had been defined earlier by both the partition of a number of phenotypic traits (Table 2) and the first DNA–DNA hybridizations (Table 3). By PFGE, the genome size of strain 27T, the serovar 3 standard and proposed type strain of *U. parvum*, had been estimated as ~760 kbp (Robertson et al., 1990). Recently, its genome was determined to contain 751719 bp (Glass et al., 2000), within 1% of that estimate. Differences in genome sizes were the first, clearly recognizable macromolecular difference between the two biovars, and led to the introduction of the parvo and T960T descriptors of the biovars (see Table 1, footnote §).

**Conserved gene sequences**

Clear evidence of the evolutionary divergence between the biovars accompanied the discovery of the third genotypic parameter of ureaplasma biovar-differentiation, the variable sequences of highly conserved gene sequences. The phylogenetic division indicated by the 16S rRNA gene data (Robertson et al., 1993, 1994) has been confirmed by the sequences of the urease (Blanchard, 1990; Teng et al., 1994) and the multiple-banded antigen genes (Teng et al., 1994) as well as by the sequence patterns of the 16S–23S rRNA intergenic spacer regions (Harasawa et al., 1996; Harasawa & Kanamoto, 1999). All have been substantiated (Kong et al., 1999). We note that although Kamla et al. (1996) have advanced the argument that phylogeny based upon the sequences of elongation factor Tu better delineated relationships among the *Mollicutes* than did the 16S rRNA tree, at present these sequences are available only for serovars 3 (Glass et al., 2000) and 14 (Kamla et al., 1996). Both are serovars of the parvo biovar; they differ by a single adenosine in a string of adenosines.

Although both the DNA–DNA hybridization and genome size analyses provided dependable parameters for discriminating between biovars, like many of the
phenotypic traits, they were impractical for application in clinical settings. However, knowledge of the 16S rRNA sequences of the biovar-representative strains brought the benefit of PCRs which identified, to biovar level, both laboratory-adapted strains and wild-type isolates (Robertson et al., 1993). When the 16S rRNA sequences for five of the 14 serovars were compared, the variance was $\leq 0.3\%$ among serovars within either biovar but $\geq 1.1\%$ between the two biovars (Robertson et al., 1994). Temporarily, it was questioned whether $\sim 1\%$ heterogeneity between 16S rRNA gene sequences was compatible with separate species identity. Fox et al. (1992) upheld the superiority of hybridization data to determine species status and persuasively argued that 16S rRNA sequences were 'more appropriate for determining inter- and intragenic relationships than taxonomic decisions'. However, the ICSB/STM, while acknowledging the justification for a second Ureaplasma species for strains from humans, advised awaiting further evidence of the efficacy of molecular differentiation methods (ICSB/STM, 1993) before agreeing, in principle, to the separation of the initial U. urealyticum into two species (ICSB/STM, 1997). Kong et al. (1999) have shown the 16S rRNA genes to be the most highly conserved of the four (previously identified) variable regions of these genomes. On the basis of their data, the maximum variance in the 16S rRNA gene sequences for all 14 serovars is 0.97%, as opposed to the 4-5% value for the 16S–23S rRNA spacer regions, 6.2–24.4% for the urease gene subunits and adjoining spacer regions, and 26–0% for the 5’-end of the multiple-banded antigen genes and 41–0% upstream of the multiple-banded antigen genes. The multiple-banded antigen genes code for putative virulence proteins on the surfaces of ureaplasma membranes (Watson et al., 1990) and exhibit serovar-related heterogeneity (Teng et al., 1994; Kong et al., 1999).

Conclusions

Altogether, we have identified at least 14 biovar-defining parameters. With a single exception (Thirkell et al., 1990), all studies cited here (Tables 2 and 3) have examined both proposed type strains and multiple, representative strains of each biovar, if not the complete set of serovar standards. Eight of the 14 parameters have been confirmed independently. These are the numerous examples of biovar-defining, protein/polypeptide patterns, including the specific urease polymorphism (Table 2), and the DNA–DNA hybridization and RFLP patterns and the heterogeneity of the four genes which have been sequenced (Table 3). The comprehensive and consistent data presented in Tables 1, 2 and 3 both fulfil and exceed the present minimal standards criteria established by the ICSB/STM (1995) to guide the taxonomy of Mollicutes. They represent a compelling argument that ureaplasmas of the parvo biovar differ sufficiently from the T960T biovar of U. urealyticum to justify separate taxa, a distinction which will better serve the needs of the scientific community.

Table 4 provides a protocol for the identification of the ureaplasmas from humans for clinical and for taxonomic purposes. Descriptions of the proposed new species, U. parvum, and the emended U. urealyticum follow.

Description of Ureaplasma parvum Robertson et al. 2001

Ureaplasma parvum (par’vum. L. neut. adj. parvum small, referring to the fact that its genome is significantly smaller than that of U. urealyticum).

Phenotypic characteristics.

Growth inhibition of cells by (or colony reaction with) polyclonal antisera to one or more of the specific antigenic determinants associated with the present parvo biovar of the human ureaplasmas (serovars 1, 3, 6 or 14) but not to any antigenic determinants associated with the emended U. urealyticum (T960T biovar of the human ureaplasmas or its serovars 2, 4, 5, 7, 8, 9, 10, 11, 12 or 13). Parvo biovar-specific polypeptide patterns obtained by PAGE: these may be enhanced through reaction with polyclonal antiserum or monoclonal antibodies to the antigenic specificities associated with the species. Growth is inhibited temporarily in the presence of 1 mM Mn$^{2+}$.

Genotypic characteristics.

Genomes of $\sim 760$ kbp demonstrate $>70\%$ hybridization patterns with the DNA of other strains of the present parvo biovar of ureaplasmas. RFLP patterns which are similar to those characteristic of other serovars of the parvo biovar but differ from those characteristic of the emended U. urealyticum: amplification by PCRs for parvo biovar-specific nucleotide sequences for the urease-associated genes, the 16S rRNA genes, the 16S–23S rRNA intergenic regions, and the 5’ end of the genes of the multiple-banded antigens, and the parvo biovar-specific RAPD PCR. The type strain of U. parvum will be the serovar 3 standard (strain 27$^T$) represented by ATCC 27815$^T$ and NCTC 11736$^T$. The G+C content of the DNA is 26 mol%; its genome size is 751 719 kbp. The most recent GenBank accession numbers for the sequences of its 16S rRNA and 16S–23S rRNA intergenic spacer region are AF073456 and AF059323, respectively. The genome sequence carries US patent number 5728522 (the annotated sequence may be found at http://genome.microbio.uab.edu/uu/ugen.htm).

Emended description of Ureaplasma urealyticum (Shepard et al. 1974) Robertson et al. 2001

Phenotypic characteristics.

Growth inhibition of cells by (or colony reaction with) polyclonal antisera to one or more of the specific antigenic determinants associated with the present T960T biovar of the human urea-
plasmas (serovars 2, 4, 5, 7, 8, 9, 10, 11, 12 or 13) but not to any antigenic determinants associated with the proposed *U. parvum* (parvo biovar of human ureaplasmas or its serovars 1, 3, 6 or 14). T960† biovar-specific polypeptide patterns obtained by PAGE: these may be enhanced through reaction with polyclonal antiserum or monoclonal antibodies to the antigenic specificities associated with the species. Growth is inhibited permanently in the presence of 1 mM Mn²⁺, with the exception of serovar 13, which shows severely delayed growth – a response which is intermediate between that shown by strains of the T960† and parvo biovars of human ureaplasmas.

**Genotypic characteristics.** Genomes ranging from ~840 to ~1140 kbp which demonstrate >70% hybridization patterns with DNA of other strains of the present T960† biovar of ureaplasmas; RFLP patterns similar to those of other serovars of the T960† biovar but which differ from those characteristic of the proposed *U. parvum*; amplification with PCRs for T960† biovar-specific nucleotide sequences for the urease-associated genes, the 16S rRNA genes, the 16S–23S rRNA intergenic regions, and the 5′-end of the genes of the multiple-banded antigens, and T960†-specific randomly amplified polymorphic DNA PCR.

The type strain of *U. urealyticum* will continue to be serovar 8 standard (strain T960†), represented by ATTC 27618T and NCTC 10177T. The estimated G + C content of the DNA is 27–28 mol%; its genome size is ~890 kbp. The most recent GenBank accession numbers for the sequences of its 16S rRNA and 16S–23S rRNA intergenic spacer region are AF073450 and AF059330, respectively.

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**REFERENCES**


Ureaplasmas of humans


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