Uroleptus willii nov. sp., a euplanktonic freshwater ciliate (Dorsomarginalia, Spirotrichea, Ciliophora) with algal symbionts: morphological description including phylogenetic data of the small subunit rRNA gene sequence and ecological notes*

Bettina SONNTAG, Michaela C. STRÜDER-KYPKE & Monika SUMMERER

Abstract: The euplanktonic ciliate *Uroleptus willii* nov. sp. (Dorsomarginalia) was discovered in the plankton of the oligo-mesotrophic Piburgersee in Austria. The morphology and infraciliature of this new species were studied in living cells as well as in specimens impregnated with protargol and the phylogenetic placement was inferred from the small subunit ribosomal RNA (SSrRNA) gene sequence. In vivo, *U. willii* is a grass-green fusiform spirotrich of 100–150 µm length. It bears about 80–100 symbiotic green algae and builds a lorica. *Uroleptus willii* is a frequent species in the summer ciliate assemblage in the upper 12 m of Piburgersee with a mean abundance of about 170 individuals l^{-1} from May through November. The algal symbionts of this ciliate are known to synthesise ultraviolet radiation – absorbing compounds. At present, the taxonomic position of *Uroleptus* has not yet been solved since the morphological features of the genus agree well with those of the Urostyloidea, while the molecular analyses place the genus within the Oxytrichidae. *Uroleptus willii* follows this pattern and groups unambiguously with other *Uroleptus* species. We assign our new species to the Dorsomarginalia BERGER, 2006. However, this placement is preliminary since it is based on the assumption that the genus *Uroleptus* and the Oxytrichidae are both monophyletic taxa, and the monophyly of the latter group has still not been confirmed by molecular data.

Key Words: *Chlorella*, ciliate plankton, freshwater, mixotrophy, mycosporine-like amino acids, phylogeny, SSrRNA gene sequence, taxonomy.

Introduction

The important role of planktonic ciliates in the microbial food web of lakes is undisputable as they contribute considerably to the biomass turnover and the production rate (e.g., CARRIAS et al. 1998; WEISSE & MÜLLER 1998; SONNTAG et al. 2006). Most ciliates in the plankton live as heterotrophs while at times a considerable part of the assemblage is mixotrophic (DOLAN 1992; JONES 1994). Mixotrophy is achieved either by a mutualistic relationship between a ciliate and symbiotic algae or the ciliates sequester the chloroplasts ('kleptoplasts') from ingested algae (e.g., DOLAN 1992). Both associations are common among mixotrophic planktonic ciliates. Most of the ciliates that bear complete algal symbionts can be detected throughout the warm season in the uppermost meters of a lake (e.g., STRÜDER-KYPKE 1999; WOELFL & GELLER 2002; MODENUTTI et al. 2005; SONNTAG et al. 2006). Moreover, such ciliates have also been observed at the oxic-anoxic boundary in a productive freshwater pond (BERNINGER et al. 1986; FINLAY et al. 1987). In general, mixotrophy is a benefit for the algae as well as for the ciliates as they can sustain periods of low food supply by nutrients received from their algal counterparts. Recently, SONNTAG et al. (2007) and SUMMERER et al. (2008) observed that the symbionts in several algal-bearing freshwater ciliates synthesised specific ultraviolet sunscreen compounds. In the oligo-mesotrophic Piburgersee, about 20 species bearing algal symbionts can be found in the summer ciliate assemblage (SONNTAG et al. in prep.). One of those 'grass-green' species is the hypotrich *Uroleptus willii* nov. sp. The Hypotricha are usually benthic in limnetic and marine habitats but are also common in soil and moss (for reviews, see FOISSNER et al. 1991, 2002; BERGER 1999, 2006). By contrast, they are rather unusual for lake plankton

* The authors would like to dedicate this paper to Prof. Wilhelm FOISSNER on the occasion of his 60th birthday in recognition of his contribution to protozoology, protistan taxonomy and teaching.

where oligotrichs and prostomatids are most common and numerous (e.g., MÜLLER 1989; WEISSE & MÜLLER 1998; FOISSNER et al. 1999; PFISTER et al. 2002; SONNTAG et al. 2006). In vivo, hypotrichs can be easily recognised as a group by their characteristic lanceolate or fusiform shape and the prominent adoral zone of membranelles (FOISSNER et al. 1991). Nonetheless, their identification is often rather difficult and requires silver impregnation. Reliable keys and reviews are already available for many species (FOISSNER et al. 1991; BERGER 1999, 2006).

Materials and methods

Uroleptus willii is frequently found in the summer months in the surface plankton of the oligo-mesotrophic Piburgersee where the type material was collected in the summer of 2004. Piburgersee is a softwater lake located at 913 m above sea level in the Austrian Central Alps (area: 13.4 ha, z_{max}: 24.6 m, catchment area: 265 ha, pH 7.2). This meromictic mountain lake is usually ice-covered from December through April (for details see TOLOTTI & THIES 2002). Additional populations of *U. willii* were observed from two lakes, Traunsee and Lake Constance. Traunsee is a 200 m deep oligotrophic lake characterised by an artificial enrichment of chloride in the hypolimnion caused by waste disposal from soda and salt industries (for details see JAGSCH et al. 2002; SONNTAG et al. 2002, 2006). Lake Constance is a deep meso-eutrophic prealpine lake where several studies on the planktonic ciliate assemblage were conducted in the north-western part ('Überlinger See') by MÜLLER and co-workers (e.g., MÜLLER 1989, MÜLLER et al. 1991).

Morphological details were studied in vivo with an Olympus BX50 microscope at magnifications of up to × 1000. Characteristics subject to change under coverglass pressure, like cell shape and movement, were studied in uncovered, swimming specimens using magnifications between ×100 and ×200. The infraciliature and various other cytological details were revealed with protargol (SKIBBE 1994; FOISSNER et al. 1999; PFISTER et al. 1999). Terminology is mainly according to BERGER (2006).

Counts and measurements on silver impregnated specimens were performed at a magnification of ×1000. In vivo measurements were conducted at magnifications of ×100 to ×1000. Although these provide only rough estimates, it is convenient to give such data as specimens usually shrink in preparations and/or become distorted during fixation. Standard deviation and coefficient of variation were calculated. Drawings of live specimens were based on free-hand sketches and micrographs, those of impregnated cells were made with a camera lucida.

The ultrastructure of the algal symbionts was studied by Transmission Electron Microscopy. Algal cells were concentrated in 2-ml reaction tubes by centrifugation (2000 g, 5 min). After fixation with glutaraldehyde and osmium tetroxide (after SHIGENAKA et al. 1973), samples were dehydrated in a graded series of acetone and embedded into low viscosity resin after SPURR (1969). Ultrathin sections (70 nm) were mounted on Formvar® (SPI Supplies, West Chester, USA) – coated grids and contrasted with sterile uranyl acetate for 15 min and sterile lead citrate for 10 min (REYNOLDS 1963). Sectioned material was observed and photographed with a Zeiss TEM 902 electron microscope.

For the molecular studies, 100 cells of *U. willii* were fixed in 80 % ethanol. DNA was extracted following the modified Chelex extraction described by STRÜDER-KYPKE & LYNN (2003). 100 µl of 5 % Chelex® 100 (Sigma, Oakville, ON, Canada) and 10 µl of Proteinase K (50 mg ml^{-1}; Epicentre, Madison, WI, USA) were added to the pelleted cells. Five microlitres of the supernatant were used in the subsequent PCR reactions. The PCR amplification was performed in a Perkin-Elmer Gene Amp 2400 thermocycler (PE Applied Biosystems, Mississauga, ON, Canada), using the internal forward primer 300F (5'-AGGGTTCGATTCCGGAG-3', ELWOOD et al. 1985) and the universal reverse primer B (5'-TGATCCTTCTGCAGGTTCACCTAC-3', MEDLIN et al. 1988). The PCR products were excised from agarose gels and purified using the MinElute Kit (Qiagen, Mississauga, ON, Canada) following the manufacturer's protocol. DNA was sequenced in both directions with a 3730 DNA Analyzer (Applied Biosystems Inc., Foster City, California, USA), using ABI Prism BigDye Terminator (ver. 3.1) and Cycle Sequencing Ready Reaction kit.

The sequence fragments were imported into Sequencher ver. 4.0.5 (Gene Codes Corp.), trimmed at the ends, assembled into contigs, and checked for sequencing errors. Sequences were aligned using the Dedicated Comparative Sequence Editor (DCSE) (DE RIJK & DE WACHTER 1993) with attention paid to secondary structural features of the molecule. The nucleotide sequences used in this article are available from the GenBank/EMBL databases and their accession numbers are given in Fig. 3. Hypervariable positions were excluded from the file prepared for phylogenetic analysis, thus resulting in a data set that comprised 1819 nucleotide positions. Missing nucleotides at the beginning or end of sequences were treated as missing by MrBayes and PAUP and gaps within the alignment were regarded as a fifth character state.

For the Bayesian inference analysis, MrModeltest ver. 2.2 (NYLANDER 2004) was employed to find the

model of DNA substitution that best fits our data. The General-Time-Reversible (GTR) model for nucleotide substitution, considering invariable sites and gamma distributed substitution rates among sites, was the best model. This model (n = 6, rates = invgamma) was implemented in MrBayes ver. 3.1.2, a phylogenetic program employing Bayesian Inference (RONQUIST & HUELSENBECK 2003), which we used to infer a phylogenetic tree (BI). Two parallel runs were performed and the maximum posterior probability of a phylogeny out of 1,000,000 trees, approximating it with the Markov Chain Monte Carlo (MCMC) and sampling every 50th generation, was computed, discarding the first 1000 trees as burn-in. Additionally, a maximum likelihood tree was constructed using PhyML (GUINDON & GASCUEL 2003, GUINDON et al. 2005) using the same parameters as described above. A maximum parsimony (MP) analysis was performed with PAUP* ver. 4.0b10 (SWOFFORD 2002), using 536 parsimony-informative characters, and with the tree bisection-reconnection (TBR) branch-swapping algorithm in effect. Species were added randomly (n = 5) and the data were bootstrap resampled 1000 times. PHYLIP ver. 3.67 (FELSENSTEIN 2005) was employed to construct a distance matrix, using DNADIST to calculate genetic distances with the Kimura-2-parameter model (KIMURA 1980). The distance trees were constructed with NEIGHBOR, using the Neighbor Joining (NJ) algorithm (SAITOU & NEI 1987). The data were bootstrap re-sampled 1000 times.

Results and discussion

Uroleptus willii nov. sp. (Fig. 1-3, Tab. 1)

Diagnosis: Size about 100–150 × 25–40 µm in vivo; fusiform; flexible. Usually two macronuclear nodules in midline. Cortical granules lacking, symbiotic green algae present. Usually with lorica. Midventral complex composed of about 72 cirri; right cirri of midventral pairs distinctly larger than left. On average 38 adoral membranelles, about 33 cirri each in right and left marginal row, three frontal cirri, one buccal cirrus, one cirrus behind right frontal cirrus, two frontoterminal cirri, five transverse cirri near posterior body end, five dorsal kineties, and three caudal cirri.

Type locality: Pelagial of Piburgersee, Austria (47°11'N, 10°53'E).

Type material: Eight slides (one holotype, seven paratypes) with protargol-impregnated specimens from Piburgersee have been deposited in the Oberösterreichische Landesmuseum at Linz (LI), Austria. Relevant specimens are marked by black ink circles on the cover glass. The SSrDNA sequence of *U. willii* has been deposited in GenBank (accession number EU399543). Moreover, six voucher slides from the Traunsee- and two voucher slides of the Lake Constance-populations (kindly provided by H. Müller, Konstanz, Germany) have been deposited in the museum in Linz.

Dedication: The authors dedicate this new species to Univ.-Prof. Dr. Wilhelm ("Willi") Foissner (Salzburg University, Austria) who celebrates his 60th birthday in 2008. The Traunsee-population was originally discovered by B.S. together with W.F.

Description: The holotype and several paratypes were studied from the population of Piburgersee. Although two additional protargol-stained populations were available, one from Traunsee and another one from Lake Constance (cultivated specimens), characteristics could not be exhaustively studied as the preparations were either bleached or the specimens disrupted during preparation. Nonetheless, many characteristic features agree with the Piburgersee-population and thus, we assume that all studied populations are conspecific. From the Traunsee-population also living individuals were studied.

Size variable, 100–150 × 25–40 µm in vivo, usually about 135 × 40 µm; in culture up to 250 µm in length; distinctly shrunken in protargol preparations (about 30 %); length:width ratio on average 3.8:1 in vivo, 2.8–4.6:1, on average 3.5:1 in protargol preparations (Tab. 1). Outline fusiform and often slightly sigmoidal with posterior portion narrowed and curved rightwards forming a short tail, ovoid when many centric diatoms ingested. Body very flexible but hardly contractile, does not withstand the pressure of a cover slip very well and quickly loses its shape and breaks slightly twisted about main body axis, scarcely dorsoventrally flattened, tapering in the anterior third, sometimes with conspicuous hunchback at height of contractile vacuole (Fig. 1a–c, 2a, g). Macronuclear nodules in midline; about 8 µm apart from each other; individual nodules globular to ellipsoidal, contain mainly small nucleoli; in cultivated specimen often only 1 nodule (Tab. 1). On average one micronucleus nearby or attached at each macronuclear nodule with unclear position, individual micronuclei globular to slightly ellipsoidal, 3.3 × 3.0 µm after protargol impregnation, do not always impregnate with protargol (Fig. 1c). Contractile vacuole at left cell margin at level of cytopharynx, cell margin distinctly bulged when filled and thus even recognisable at low magnification (×100; Fig. 2a). No cortical granules. Cytoplasm colourless, contains about 80–100 symbiotic green algae that are 3–6 µm across, few 1–2 µm long crystal-like structures in the posterior part, and sometimes many food vacuoles with centric diatoms; appears dark at low

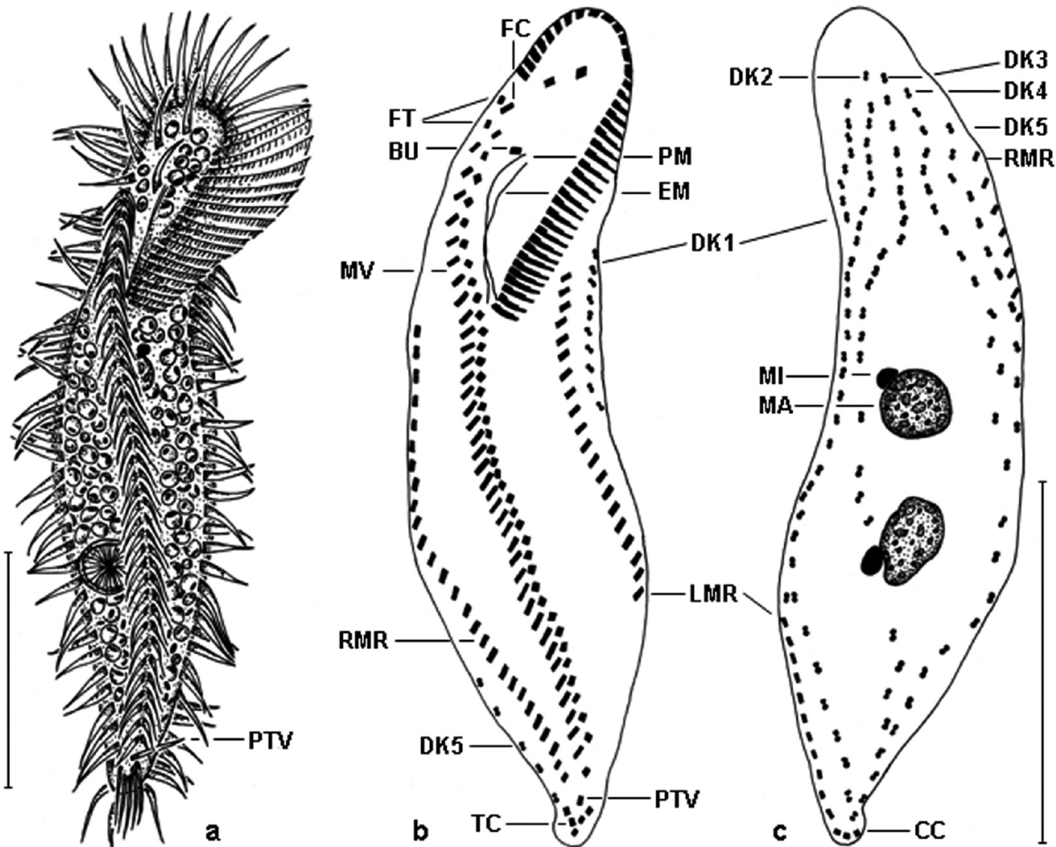

Fig. 1a–c: *Uroleptus willii*, type population from Piburgersee from life (a) and after protargol impregnation (b, c). **a**: Ventral view of a representative specimen containing many algal symbionts. **b, c**: Infraciliature of ventral and dorsal side and nuclear apparatus of holotype specimen (algal symbionts not illustrated). BU – buccal cirrus, CC – caudal cirri, DK1-5 – dorsal kineties 1-5, EM – endoral membrane, FC – right frontal cirrus, FT – frontoterminal cirri, LMR – left marginal row, MA – macronuclear nodule, MI – micronucleus, MV – midventral complex, PM – paroral membrane, PTV – ventral cirrus ahead of transverse cirri, RMR – right marginal row, TC – transverse cirri. Scale bars 50 µm.

magnifications (×40–100; Fig. 1a, 2a–e). Mucocysts present, ejected when methyl-green pyronin is added. Lorica tubular, inside smooth and more or less as wide as the ciliate, hyaline, elastic, consists of mucus covered with detritus, often colonised by bacteria and small protists, open on one or both ends (Fig. 2b). Movement without peculiarities, that is, swims rather slowly while rotating about main body axis, performs fast backward movement when stressed; within lorica more or less immobile, only short forward and backward movements while grazing, twisting and turning inside lorica when disturbed or stressed, individuals without lorica sometimes 'tremble' (probably onset of building a new lorica?).

Cirral pattern and number of cirri of usual variability (Fig. 1b, c, Tab. 1). Most cirri about 12–13 µm long in vivo and of similar size, except left cirri of midventral pairs which are distinctly smaller than remaining cirri. Marginal rows end subterminal, right row commences near level of buccal cirrus. Frontal cirri slightly enlarged, form oblique row with right cirrus, as is usual, behind distal end of adoral zone. Buccal cirrus right of anterior end of undulating membranes and near level of anterior end of right marginal row. Midventral cirri only indistinctly zigzagging, midventral complex terminates ahead of transverse cirri, right cirri of midventral pairs enlarged as usual. Transverse cirri subterminal, about half as wide as marginal cirri, hardly recognizable both in vivo and after protargol impregnation; one pretransverse cirrus, flexible and more or less erected from the body in an angle of about 90° in vivo (Fig. 1a-c, 2c, f, g).

Dorsal bristles about 4 µm long in vivo, about 5 µm at the posterior body end, arranged in five rows usually not easily recognisable both in vivo and in protargol preparations, in the anterior portion arranged more closely to each other than in the posterior portion of the cell (Fig. 1c, 2c). Caudal cirri appear inelastic in vivo, about as long as marginal and transverse cirri, difficult to recognise even in protargol preparations.

Adoral zone occupies 30–44 %, on average 37 % of body length and of usual shape and structure; composed of an average of 38 membranelles, bases of large membranelles up to 5–6 µm wide in vivo. Buccal cavity moderately flat and wide, lip inconspicuous (Fig. 1a-c, 2a, g, Tab. 1).

Occurrence and ecology: To date found at type location, i.e., Piburgersee (Tyrol, Austria) and in Traunsee, Austria (47°51'N, 13°47'E) and Lake Constance, Germany (47°38'N, 9°22'E). *Uroleptus willii* is well adapted to the euplanktonic life. In the upper twelve meters of Piburgersee, the species can be detected from May through November in mean numbers of about 170 individuals l^{-1} with a maximum of about 1700 cells l^{-1} in September. The Traunsee-population was observed in

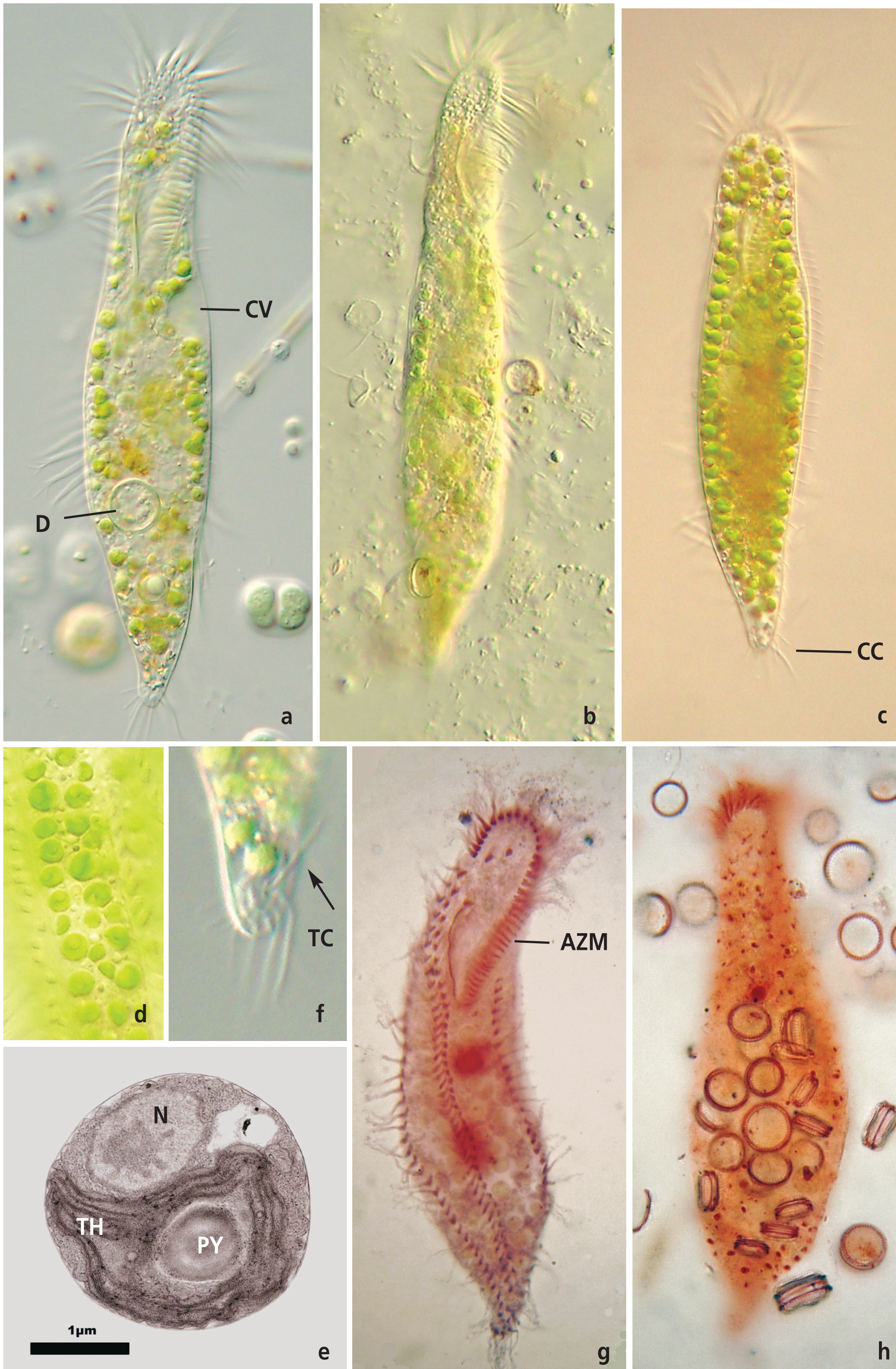

Fig. 2a–h: *Uroleptus willii* from life (a–d, f), in the TEM (e) and after protargol impregnation (g, h). **a–c**: Ventral views showing, inter alia, the contractile vacuole and an ingested diatom (a), the ciliate within the hyaline lorica (b), and the dorsal bristles and caudal cirri (c). **d, e**: Overview of the grass-green algal symbionts (d) and ultrathin section (e). **f**: Ventral view showing the transverse cirri and the pretransverse ventral cirrus (arrow). **g, h**: Ventral cirral pattern with the midventral complex (g) and dorsal view with many ingested diatoms (h). AZM – adoral zone of membranelles, CC – caudal cirri, CV – contractile vacuole, D – diatom, N – nucleus, PY – pyrenoid, TC – transverse cirri, TH – thylacoide membranes.

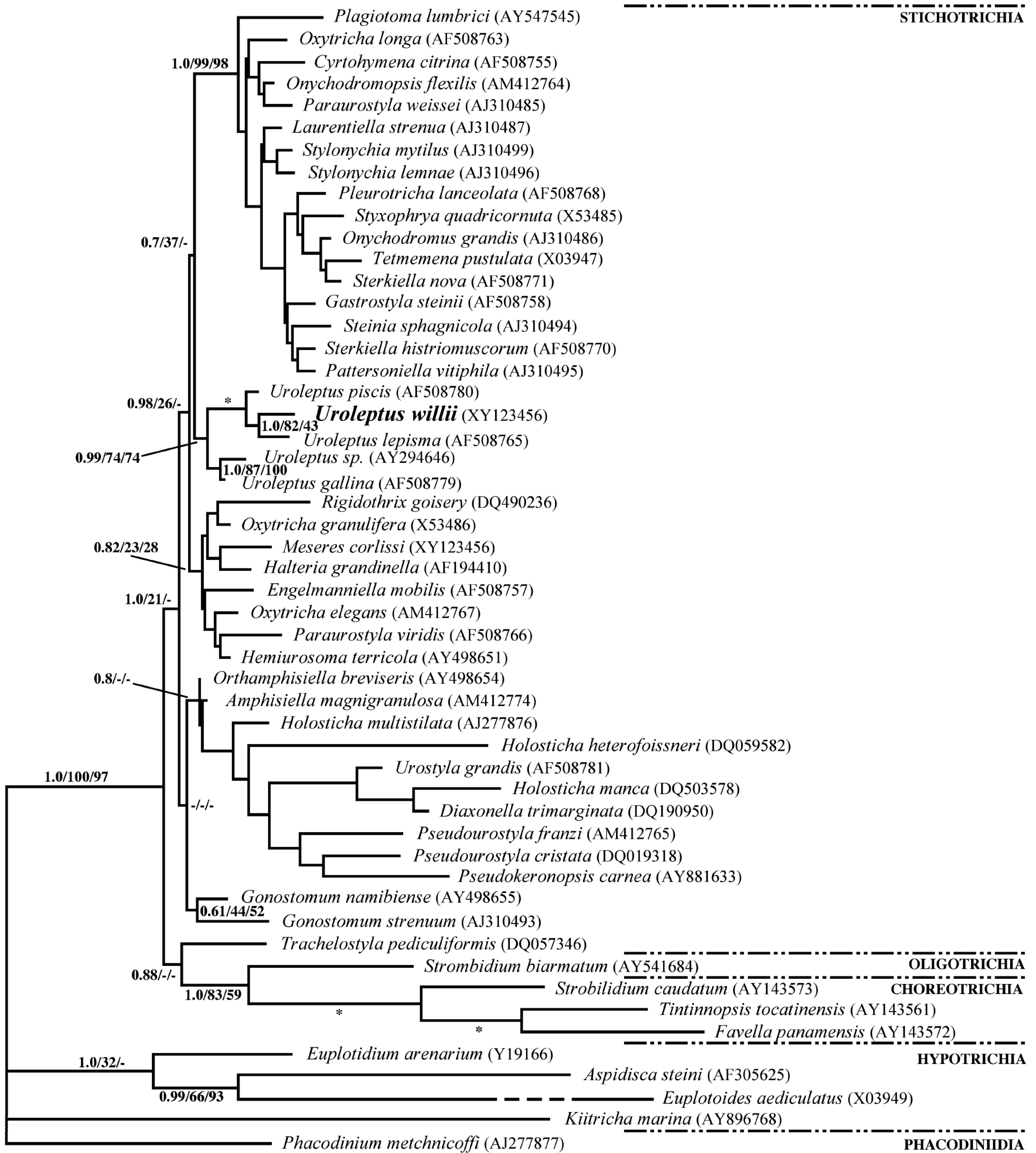

Fig. 3: Maximum likelihood tree computed with PhyML (Guindon & Gascuel 2003, Guindon et al. 2005), based on the General Time-reversible (GTR) model with gamma-distribution and an estimate of invariable sites, determined by MrModeltest (Nylander 2004). The first numbers at the nodes represent the posterior probability values of the Bayesian analysis (BI, Ronquist & Huelsenbeck 2003) and the second and third numbers represent bootstrap values (percent out of 1000 replicates) for maximum parsimony (MP, Swofford 2002) and neighbor joining (NJ, Saitou & Nei 1987), respectively. Due to the low support at most nodes, only the support values of the major branches are shown. An asterisk represents full support in all three analyses and dashes indicate bootstrap values of less than 20 % in MP and NJ. The scale bar represents five substitutions per 100 nucleotides. The new sequence appears in bold face.

Table 1: Morphometric data on *Uroleptus willii* from Piburgersee.

Characteristics[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	103.2	101	12.2	2.7	11.8	82	130	21
Body, width	29.3	30	3.6	0.8	12.2	24	37	21
Body length:width, ratio	3.5	3.5	0.4	0.1	11.9	2.8	4.6	21
Anterior body end to proximal end of adoral zone, distance	38.1	38	3.5	0.8	9.2	32	45	21
Body length:length of adoral zone, ratio	2.7	2.6	0.3	0.1	11.4	2.3	3.3	21
Anterior body end to paroral membrane, distance	17.7	18	1.8	0.4	10.2	15	21	21
Anterior body end to endoral membrane, distance	18.3	18	1.8	0.4	10.1	15	22	21
Paroral membrane, length	17.9	18	2.3	0.5	12.7	13	23	21
Endoral membrane, length	17.7	18	2.5	0.5	14.1	12	24	21
Anterior body end to buccal cirrus, distance	17.3	17	1.8	0.4	10.2	15	21	21
Anterior body end to first frontoterminal cirrus, distance	16.1	16	1.4	0.3	8.9	13	19	21
Anterior body end to second frontoterminal cirrus, distance	18.6	19	1.5	0.3	8.2	15	22	21
Anterior body end to right marginal row, distance	22.0	23	2.4	0.5	10.8	18	27	21
Anterior body end to anterior macronuclear nodule, distance	43.8	42	4.3	0.9	9.9	38	55	21
Nuclear figure, length	30.0	30	5.1	1.1	17.1	22	39	21
Anterior macronuclear nodule, length	10.0	10	1.2	0.3	11.9	8.5	13	21
Anterior macronuclear nodule, width	8.5	8	0.7	0.1	7.9	7.5	10	21
Distance between macronuclear nodules	8.3	8	3.0	0.7	36.2	3	15	21
Macronuclear nodules, number	2.0	2	0.0	0.0	0.0	2	2	21
Anterior micronucleus, length	3.3	3	0.4	0.1	13.1	3	4	21
Anterior micronucleus, width	3.0	3	0.0	0.0	0.0	3	3	21
Micronuclei, number	2.0	2	0.8	0.2	39.3	1	5	21
Adoral membranelles, number	38.3	38	3.1	0.7	8.0	33	47	21
Frontal cirri, number	3.0	3	0.0	0.0	0.0	3	3	21
Buccal cirri, number	1.0	1	0.0	0.0	0.0	1	1	21
Frontoterminal cirri, number	2.0	2	0.0	0.0	0.0	2	2	21
Cirri behind right frontal cirrus, number	1.0	1	0.0	0.0	0.0	1	1	21
Midventral complex, number of cirral pairs	36.0	36	5.0	1.1	14.0	30	51	21
Midventral cirri, number [b]	72.0	72	10.1	2.2	14.0	60	102	21
Transverse cirri, number	5.0	5	0.0	0.0	0.0	5	5	3
Caudal cirri, number	3.0	–	–	–	–	–	–	1
Right marginal cirri, number	32.8	32	3.8	0.8	11.7	28	45	21
Left marginal cirri, number	32.9	32	4.2	0.9	12.9	27	47	21
Dorsal kineties, number	5.0	5	0.0	0.0	0.0	5	5	18
Zoochlorellae, diameter	3.3	3	0.5	0.1	15.8	2.0	4.0	21

[a] Field specimens from the same sampling date after protargol impregnation. Measurements in µm. CV – coefficient of variation in %, M – median, Max – maximum, Mean – arithmetic mean, Min – minimum, n – number of specimens investigated, SD – standard deviation, SE – standard error.
[b] Cirrus behind right frontal cirrus not included.

autumn 2000. In culture, the ciliates live in large colonies that consist of several loricae each inhabited by one individual. Phylogenetic studies of the symbionts showed that they seem to be host-specific and belong to the Trebouxiophyceae (Chlorophyta; SUMMERER et al. 2008; Fig. 2d, e). The algal symbionts of *U. willii* are able to synthesise specific ultraviolet sunscreen compounds, so-called mycosporine-like amino acids (SONNTAG et al. 2007). Furthermore, this mutualistic relationship between ciliate and algae enables *U. willii* to receive nutrients synthesised by its symbionts as well as from ingested algal food. In culture, *U. willii* grows well at about 15°C in Woods-Hole-MBL – medium (GUILLARD & LORENZEN 1972) under a light/dark cycle with cryptomonads as food.

Conjugating specimens were observed in June, August and September 2004 in Piburgersee. At one occasion in June 2004, the whole population of *U. willii* was heavily parasitised by suctorians (Suctoria, Ciliophora), most probably of the genus *Podophrya* sp. (SONNTAG et al. in prep.).

Phylogenetic analyses: The SSrDNA of *U. willii* has been only partially amplified. Therefore, the length of the sequence is only 1392 nucleotides. The GC content of this sequence is 46 %, identical to the other *Uroleptus* species.

Uroleptus willii clusters with the other *Uroleptus* species, showing sequence similarities of 97–98 % to its congeners (Fig. 3). The genetic distances among these species range from 0.82 % (*U. gallina* and *Uroleptus* sp.) to 3.27 % (*U. willii* and *Uroleptus* sp.). However, the monophyly of the genus is only strongly supported by the BI analysis (0.99 BI, 74 % MP, 74 % NJ). Instead, the species form two highly supported clusters (*U. gallina* and *Uroleptus* sp. on one hand [1.0 BI, 87 % MP, 100 % NJ], and *U. piscis*, *U. lepisma*, and *U. willii* on the other [1.0 BI, 100 % MP, 100 % NJ]). In the current analysis, *U. lepisma* is the sister species to *U. willii* (1.0 BI, 82 % MP, 43 % NJ) and their genetic distance is 2.12 %.

Generic assignment and comparison with related species

According to morphological features like the zigzagging midventral pattern, the genus *Uroleptus* can be assigned to the Urostyloidea (BORROR 1972). However, from molecular analyses, the genus is more closely related to the Oxytrichidae and does not cluster with the urostyloids (e.g., CROFT et al. 2003; DALBY & PRESCOTT 2004; FOISSNER et al. 2004; HEWITT et al. 2003; SCHMIDT et al. 2007). BERGER (2006) discussed this discrepancy in detail and proposed the Dorsomarginalia based on the assumption that the genus *Uroleptus* and the Oxytrichidae are both monophyletic taxa. Our morphological and phylogenetic data confirm what has been found so far. The monophyly of the Oxytrichidae is not supported by molecular data and, therefore, one of the assumptions for the erection of the Dorsomarginalia cannot be confirmed. However, the support values within the clusters are very low and the topologies of the trees inferred by different phylogenetic algorithms (ML, BI, MP, NJ) are also not consistent. At this point, we follow BERGER's (2006) suggestion and assign the new species *U. willii* to the Dorsomarginalia. Future comprehensive studies are needed to validate or reject this taxon.

Morphologically, the euplanktonic *U. willii* can be easily identified and distinguished from all other congeners by its grass-green appearance caused by its algal symbionts. However, in vivo, *U. willii* can be confused with *Stichotricha secunda* PERTY, 1849 as the overall appearance is very similar and *S. secunda* is often found at the same time and in the same water depths as *U. willii* in Piburgersee (FRIED 1995; SONNTAG et al. in prep.). Both species are of about the same size, bear algal symbionts and build a lorica. Nonetheless, the shape of *U. willii* is fusiform vs. slenderly fusiform in *S. secunda* and the buccal field occupies about 37 % of body length and the anterior end is rounded vs. 40–50 % and acuminate. In addition, *Uroleptus willii* and *S. secunda* differ in the cirral pattern (midventral complex and two marginal rows only slightly spiralling vs. four rows strongly spiralling). Protargol impregnations are recommended for reliable identification, especially for inexperienced workers. However, if a specimen has the following features in vivo, then it is likely *U. willii*: size about 100–150 × 30–40 μm, fusiform and slightly sigmoid; buccal field moderately flat and wide; one buccal cirrus, midventral complex present, five transverse cirri and one pretransverse ventral cirrus, three inelastic caudal cirri.

Uroleptus willii cannot be confused with other euplanktonic hypotrichs as these either do not bear algal symbionts or have remarkable shapes or both, i.e., *Hypotrichidium conicum* ILOWAISKY, 1921, *Pelagotrichidium faurei* (TUFFRAU, 1972) JANKOWSKI, 1978, *Pseudostrombidium planctonicum* HORVÁTH, 1933 and *Spiretella plancticola* (GELEI, 1933) BORROR, 1972. Other euplanktonic ciliates with algal symbionts can also hardly be confused with *U. willii* as they have very different shapes and/or sizes (see FOISSNER et al. 1999).

From our phylogenetic results, *U. willii* clusters well with the other species of the genus *Uroleptus* that have been sequenced so far, i.e., *Uroleptus gallina* (MÜLLER, 1786) FOISSNER, BLATTERER, BERGER, KOHMANN, 1991, *U. lepisma* (WENZEL, 1953) FOISSNER, 1998, *U. piscis* (MÜLLER, 1773) EHRENBERG, 1831, and one unidentified species (Fig. 3). However, the closest congener is *U. lepisma*, which is 90–110 μm (type population) or up to 160–200 μm (two populations observed by BERGER & FOISSNER 1989) long (vs. 100–150 μm in *U. willii*), does not bear algal symbionts, has transverse and caudal cirri that are longer than the marginal cirri (vs. of about same length), has dorsal bristles that are 7 μm long (vs. 4–5 μm), and lives in *Sphagnum* mosses or soil (WENZEL 1953; BERGER & FOISSNER 1989). Additionally, the genetic distance of 2.12 % between these two is large enough to separate them at the species level. Other *Uroleptus* species that are similar to *U. willii* from their size and shape do not contain algal symbionts, i.e., *U. gallina* and *U. musculus* (KAHL, 1932) FOISSNER, BLATTERER, BERGER & KOHMANN, 1991. Although FOISSNER (1980) and KRAINER (1988) mentioned the possible presence of symbiotic algae in *U. caudatus* (STOKES, 1886), the size (120–350 μm), contractility and details of the infraciliature (e.g., distance of transverse cirri from posterior end [10 μm]; no pretransverse ventral cirri) do not resemble *U. willii*.

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Addresses of authors:

Dr. Bettina SONNTAG
Dr. Monika SUMMERER
Lab of Plankton Ecology & Aquatic Photobiology
Institute of Ecology
University of Innsbruck
Technikerstrasse 25
6020 Innsbruck
Austria
E-mail: bettina.sonntag@uibk.ac.at
(corresponding author)
monika.summerer@uibk.ac.at

Dr. Michaela C. STRÜDER-KYPKE
Department of Integrative Biology
College of Biological Science
University of Guelph
Guelph, Ontario
Canada, N1G2W1
E-mail: mstruede@uoguelph.ca