Characterization and localization of *Rickettsia* sp. in water beetles of genus *Deronectes* (*Coleoptera: Dytiscidae*)

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**Abstract**

In the present study, *Rickettsia* sp. was detected in four water beetles of the genus *Deronectes* (*Dytiscidae*) for the first time. Rickettsiae were found in 100% of examined specimens of *Deronectes platynotus* (45/45), 39.4% of *Deronectes aubei* (28/71), 40% of *Deronectes delarouzei* (2/5) and 33.3% of *Deronectes semirufus* (1/3). Analysis of 16S rRNA gene sequences revealed a phylogenetic relationship with rickettsial isolates of *Limonia chorea* (*Diptera*), tentatively classified as members of the basal ancestral group. Phylogenetic analysis of the gltA (citrate synthase) gene sequences showed that *Deronectes* symbionts were closest to bacterial symbionts from spiders. Ultrastructural examinations revealed typical morphological features and intracellular arrangements of rickettsiae. The distribution, transmission and localization of *Rickettsia* sp. in *D. platynotus* were studied using a diagnostic PCR assay and FISH. Eggs from infected females of *D. platynotus* were all *Rickettsia*-positive, indicative of a vertical transmission.

**Introduction**

Gram-negative bacteria of the genus *Rickettsia* belong to the family *Rickettsiaceae* in the subdivision of the monophyletic order *Rickettsiales* (*Alphaproteobacteria*). All members of the genus *Rickettsia* are obligate intracellular bacteria that reside free in the cytoplasm of eukaryotic cells. Mostly known as pathogenic endosymbionts of arthropods (ticks, mites, fleas and lice), rickettsiae cause human and animal diseases with a worldwide distribution (Eremeeva & Dasch, 2000). Some species are also capable of infecting and causing disease in plants (Davis *et al.*, 1998). Rickettsiae are usually transmitted by arthropods to vertebrates via feces or salivary secretions. Because of their considerable medical importance, the genus *Rickettsia* was traditionally divided into two groups (Weiss & Moulder, 1984): the spotted fever group, including *Rickettsia rickettsii* and allied species, causative agents of spotted fever; and the typhus group, including *Rickettsia prowazekii* and *Rickettsia typhi*, the agents of murine and endemic typhus, respectively. In recent years, new rickettsiae have been discovered that do not belong to the previously described subgroups. A third group named ‘ancestral group’, partially subdivided into the bellii, leech and limoniae groups (Perlman *et al.*, 2006; Perotti *et al.*, 2006), was established (Stothard & Fuerst, 1995; Fournier & Raoult, 2007), and consists of *Rickettsia bellii*, *Rickettsia canadensis* and additional numerous rickettsial isolates that have been isolated from different terrestrial arthropods such as whiteflies, flies, aphids and beetles (Chen *et al.*, 1996; Fukatsu *et al.*, 2000; Campbell *et al.*, 2004; Gottlieb *et al.*, 2006; Zchori-Fein *et al.*, 2006). The ancestral group is still an initial theoretical classification that is not based on morphological criteria or biochemical features concerning basal standing rickettsial isolates in the genus. Rickettsiae of the ancestral group were not shown to be pathogens for humans or their animal hosts. However, insects are not the only vector of rickettsiae. Recently, rickettsiae have been found in spider mite *Tetranychus urticae* (Hoy & Jeyaprakash, 2005), various spider species (Goodacre *et al.*, 2006), amoeba *Nuclearia pattersoni* (Dykova *et al.*, 2003), marine ciliate *Diophrys appendiculata* (Vannini *et al.*, 2005) and several leeches from freshwater environments (Kikuchi & Fukatsu, 2005; Gottlieb *et al.*, 2006), which also cluster into the ancestral group. Analyses of 16S rRNA genes suggest that many of these rickettsial isolates could represent new genera within the order *Rickettsiidae*. As already supposed (Yu & Walker, 2006), the family *Rickettsiaceae* could be more widespread than observed so far.
The purpose of this study was to investigate and identify symbiotic bacteria in selected water beetles of the palearctic genus Deronectes. The association of endosymbiotic bacteria with water insects, especially water beetles, was already presumed in previous studies (Maddison et al., 1999; Shull et al., 2001). Representatives of the genus Deronectes are predacious and colored uniformly black or brown. Deronectes species usually live among gravel in small, swift or strongly flowing streams with sparse vegetation. Most species prefer mountainous regions, some occurring at a high altitude. To date 55 species have been described (Ferry & Brancucci, 1997; Ferry et al., 2001).

In the present molecular study, we report the first finding of a coccobacillus bacterium in Deronectes, which could be identified as a member of the genus Rickettsia. A total of 136 specimens from seven Deronectes species were examined for the existence of Rickettsia sp. using a diagnostic PCR assay. Phylogenetic analysis inferred by 16S rRNA gene and particularly the rickettsial gltA (citrate synthase) gene disclosed the phylogenetic position of beetle Rickettsia in the existing phylogenetic system of Rickettsia endosymbions. FISH was used for the investigation of rickettsial distribution and vertical transmission rates. The morphological characteristics of the bacterium were analyzed by electron microscopic observations.

**Materials and methods**

**Samples**

A total of 136 Deronectes specimens were collected between 2003 and 2008 (Table 1) by kick-sampling (Schwoerbel, 1994). Deronectes platynotus and Deronectes latus were obtained from Fichtelgebirge, a mountain range in northeastern Bavaria, Germany. Deronectes aubei was collected from Black Forest (Germany), Italy and French Alps. Deronectes delarouzei, Deronectes semirufus, D. aubei sanfilippi and Deronectes moestus inconspectus came from Spain, Italy and France. Beetles were brought to laboratory alive and embedded for histology/FISH or dissected for diagnostic PCR immediately.

### DNA extraction, cloning and sequencing

DNA was extracted using a QUIAGEN DNeasy® Tissue Kit (QUIAGEN GmbH, Germany) following the protocol for animal tissue. The eubacterial 16S rRNA gene was PCR amplified using the primer set 07F (5'-AGAGTTT GATCMTGGGTCAG-3') and 1507R (5'-TACCTGTTGAC GACTTCAC-3') (Lane, 1991). All PCR reactions were performed in a Biometra thermal cycler with the following program: an initial denaturating step at 94°C for 3 min, followed by 34 cycles of 94°C for 30 s, 50°C for 2 min and 72°C for 1 min. A final extension step of 72°C for 10 min was included. PCR products of the expected sizes were cloned using the TOPO TA Cloning® Kit (Invitrogen, CA). Suitable clones for sequencing were selected by restriction fragment length polymorphism (RFLP). Inserts were digested by restriction endonucleases HaeIII and TaqI. Plasmids containing the DNA inserts of the expected sizes were sequenced at the DNA analytics core facility of the

### Histology

Before all the beetles were fixed in 4% paraformaldehyde overnight, the elytra were carefully removed. The fixed beetles were washed in 1× phosphate-buffered saline and 96% ethanol (1:1), dehydrated serially in ethanol (70%, 90%, 2× 100%) and embedded in UNICRYL™ (Plano GmbH, Germany). Serial sections (2μm) were cut using a Leica Jung RM2035 rotary microtome (Leica Instruments GmbH, Germany), mounted on epoxy-coated glass slides and subjected to FISH.

### Table 1. Infection rates of Rickettsia sp. in natural populations of Deronectes spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Date of collection</th>
<th>No. of individuals</th>
<th>% of infection (infected/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. platynotus</em></td>
<td>Fichtelgebirge, Bavaria, Germany</td>
<td>2007/2008</td>
<td>45</td>
<td>100 (45/45)</td>
</tr>
<tr>
<td><em>D. aubei</em></td>
<td>Black Forest, Germany, Italy, France</td>
<td>2003–2008</td>
<td>71</td>
<td>39.4 (28/71)</td>
</tr>
<tr>
<td><em>D. latus</em></td>
<td>Fichtelgebirge, Bavaria, Germany</td>
<td>17.10.2007</td>
<td>7</td>
<td>0 (0/7)</td>
</tr>
<tr>
<td><em>D. delarouzei</em></td>
<td>Bonansa, El pont de Suert, Spain</td>
<td>05.10.2007</td>
<td>1</td>
<td>40 (2/5)</td>
</tr>
<tr>
<td></td>
<td>Llesp, El pont de Suert, Spain</td>
<td>10.08.2004</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saldes, Spain</td>
<td>05.10.2007</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>D. semirufus</em></td>
<td>Grotta all’Onda, Casoli, Lucca, Tuscany, Italy</td>
<td>09.10.2003</td>
<td>1</td>
<td>33.3 (1/3)</td>
</tr>
<tr>
<td><em>D. aubei sanfilippi</em></td>
<td>Sospel, Moulinet, Apuane-Maritimes, France</td>
<td>07.10.2003</td>
<td>1</td>
<td>33.3 (1/3)</td>
</tr>
<tr>
<td><em>D. moestus inconspectus</em></td>
<td>Fociomboli, Apuane Alps, Italy</td>
<td>07.09.2007</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vernet les Bains, Pyr. Orientales, France</td>
<td>07.10.2007</td>
<td>3</td>
<td>0 (0/3)</td>
</tr>
<tr>
<td></td>
<td>Saldes, Spain</td>
<td>17.10.2007</td>
<td>2</td>
<td>0 (0/2)</td>
</tr>
</tbody>
</table>
University of Bayreuth with M13 forward and M13 reverse sequencing primers (Invitrogen).

For diagnostic PCR, the 16S rRNA gene was amplified using the Rickettsia-specific primers Ri_170F ([5'-GGGCGTGTTGCTTA AATTATTTAGT-3']) and Ri_1500R ([5'-ACTGTTAGCTACC ACCTTCAGG-3']). The citrate synthase gene (gltA) was detected using the primers CS5_F ([5'-TCTTTATGGGGACCC AGCC-3']) and CS4_R ([5'-TTCCATTTGCGCACCCAGG-3']). PCR primers were designed using the probe design tool of the ARB software package (Ludwig et al., 2004). Diagnostic PCR was performed under the temperature profile described above.

**FISH**

The following probes were used for FISH targeted to the 16S rRNA gene: eubacterial probe EUB338 ([5'-GCCGTAGGAGT-3']) and NON338 ([5-]) with antibleaching solution (VECTASHIELD 225 mM NaCl, 5 mM EDTA, and 0.01% SDS], mounted rinsed in washing buffer [20 mM Tris-HCl (pH 8.0), 0.9 M NaCl, 0.01% sodium dodecyl sulfate (SDS), and 20% formamide] containing 10 pmol of fluorescent probes per milliliter, incubated at 46 °C for 20 min. Following postfixation in 2% osmium tetroxide for 20 min. Following postfixation in 2% osmium tetroxide for 90 min, rinsed in washing buffer [20 mM Tris-HCl (pH 8.0), 225 mM NaCl, 5 mM EDTA, and 0.01% SDS], mounted with antibleaching solution (VECTASHIELD® Mounting Medium; Vector Laboratories, UK) and viewed under a fluorescent microscope.

**Electron microscopy**

Male accessory glands of D. platynotus were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 1 h, embedded in 2% agarose and fixed again in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) overnight. Specimens were washed in 0.1 M cacodylate buffer three times for 20 min. Following postfixation in 2% osmium tetroxide for 2 h, the specimens were washed and stained en bloc in 2% uranyl acetate for 90 min. After fixation, specimens were dehydrated sequentially in ethanol (30%, 50%, 70%, 95% and 3× 100%), transferred to propylene oxide, and embedded in Epon. Ultrathin sections (70 nm) were cut with a diamond knife (MicroStar, Huntsville, TX) on a Leica Ultracut UCT microtome (Leica Microsystems, Vienna, Austria). Ultrathin sections were mounted on pioloform-coated copper grids, and stained with saturated uranyl acetate, followed by lead citrate. The sections were viewed with a Zeiss CEM 902 A transmission electron microscope (Carl Zeiss, Oberko-chen, Germany) at 80 kV. Micrographs were taken using an SO-163 EM film (Eastman Kodak, Rochester, NY).

**Phylogenetic analysis**

High-quality sequences of the 16S rRNA gene and the gltA gene for citrate synthase were aligned with the CLUSTALW software in BIOEDIT (Hall, 1999) and transferred to the ARB software package and edited manually. Phylogenetic analyses using maximum parsimony were performed using PAUP® version 4.0b10 (Swofford, 2000). The heuristic search included 10 000 random addition replicates and tree bisection–reconnection (TBR) branch swapping with the Multrees option. A 50% majority-rule consensus tree of the most parsimonious tree was constructed and exported to ARB program package, where branch lengths were calculated. Bootstrap analyses were performed with 500 replicates with TBR branch swapping, 25 random addition replicates and the Multrees option in PAUP®. The gltA citrate synthase sequences were checked for chimeras using CODE (Gonzalez et al., 2005).

**Sequence data**

Clone sequences of 16S rRNA gene and gltA sequences for the D. platynotus-, D. aubei-, D. semirufus- and D. delarouzei-associated Rickettsia sp. were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers FM177868–FM177878 and FM955310–FM955315, respectively.

**Results**

**Identification of bacterial symbiont**

A 1.5-kb segment of the eubacterial 16S rRNA gene was amplified by PCR and was subjected to cloning and RFLP typing. Overall, 19 clones were sequenced and compared with other sequences found in GenBank. The nucleotide sequences of clones exhibited 99% similarity to the sequence of Rickettsia limoniae (AF322442, AF322443) from cranefly L. chorea (Diptera, Limoniidae). The validity of the sequences was confirmed by FISH performed with probe Rick_B1. Furthermore, we sequenced a 416-bp fragment of the gltA gene (citrate synthase) from four Rickettsia-positive D. platynotus beetles as well as from D. aubei (404 bp), D. semirufus (367 bp) and D. delarouzei (401 bp). The gltA sequences from D. platynotus rickettsiae were 100% identical to rickettsial isolate (DQ 231491) from spider Pityophantes phyrgianus (Goodacre et al., 2006). The gltA gene sequences from rickettsial symbionts of D. aubei and D. delarouzei exhibited 99% similarity to the sequence of P. phyrgianus, whereas rickettsial gltA sequences from D. semirufus showed 98% similarity to Rickettsia endosymbionts of spider Meta menegi.
Screening for the presence of *Rickettsia* sp.

Six *Deronectes* species and one subspecies were examined using *Rickettsia*-specific primers in a diagnostic PCR assay (Table 1). A total of 45 *D. platynotus* were screened. *Rickettsia* sp. could be detected in 100% of all tested *D. platynotus*. In contrast, only 39.4% of 71 examined individuals of *D. aubei* were infected. Both species were collected in different seasons and populations. Just two individuals of *D. delarouzei* and one individual of *D. semirufus* were found to be positive for the *Rickettsia* symbiont. For elaboration of infection rates in these two species, more specimens from different populations have to be examined for rickettsiae. No rickettsiae were detected in seven individuals of *D. latus*. In the same way, three surveys of *D. aubei* sanfilippoi indicated a negative signal for *Rickettsia*. Two examined individuals of *D. moestus* inconspectus also did not show any infection of *Rickettsia*. However, only a few individuals of *D. latus*, *D. aubei* sanfilippoi and *D. moestus* inconspectus could be examined for the presence of rickettsiae. All examined species of *D. platynotus* and *D. aubei* exhibited a well-balanced sex ratio. Furthermore, there was no evidence for male killing, parthenogenesis or other sex ratio distortion in numerically examined species of *D. platynotus* and *D. aubei*. Current studies indicated that further water beetles are associated with *Rickettsia* sp. too (data not shown). Examined species of *Agabus* melanarius, *Agabus wasastjernae*, *Agabus guttatus*, *Hydroporus gyllenhalii*, *Hydroporus tristis*, *Hydroporus umbrosus* and *Hydroporus obscurus* also exhibited *Rickettsia* infections.

**In situ hybridization of *Rickettsia* symbiont**

FISH revealed a remarkable affection of the *Rickettsia* symbiont (Fig. 1a). All tissues that are in contact with the hemolymph are affected by rickettsiae. The symbionts could be identified in all compartments of *Deronectes*, including the head (ommatidia) (Fig. 1b) and legs. Actually, internal parts of elytra with soft tissue exhibited bacteria. Especially, numerous rickettsiae could be detected in tissue of active metabolism, such as the fatbody and internal reproduction organs. A lower number of rickettsiae was found in muscles. In addition, *Rickettsia* sp. is more abundant in females than males. Accessory glands of males will be affected predominantly (Fig. 1c). The strongest signals of a *Rickettsia*-specific probe were found in females of *D. platynotus*. Minor signals of symbionts were detected in *D. aubei*, *D. semirufus* and *D. delarouzei* (data not shown).

The distribution of the *Rickettsia* was also investigated in eggs and larvae of *D. platynotus*. The presence of large numbers of symbionts in oocytes and follicle cells (Fig. 1d), and the detection of *Rickettsia* in eggs (Fig. 1e) are indicative of the vertical transmission of symbionts. In the second and third larval stages of *Deronectes*, rickettsiae could be detected in the entire body. The number of symbionts increased from stage to stage.

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![Figure 1](image-url)

**Fig. 1.** FISH with specific probe Rick_B1 (Cy3) of square sections of water beetle *Deronectes platynotus* adults. (a) *Rickettsia* sp. infection of fatbody in a female abdomen. Scale bar = 40 μm. (b) Square section of head; numerous *Rickettsia* signals from ommatidia (arrows). *Rickettsia* sp. clustered around the cerebral ganglia. Scale bar = 40 μm. (c) Male accessory glands infected by intracytosolic *Rickettsia* sp. Bacteria are located near the connective tissue (arrow). Scale bar = 20 μm. (d) Ovariole infected by *Rickettsia* sp. symbiont. Scale bar = 20 μm. (e) Bacterial symbionts are also present within the egg (arrow). Surrounding tissue of ovarioles harbouring *Rickettsia*. Scale bar = 50 μm. FB, fat body; B, brain; E, egg; AGT, accessory glandular tissue; M, muscle; O, ovariole; OM, ommatidia. (a), (b) and (c) were taken by confocal microscope. (d) and (e) were taken by a conventional fluorescence microscope.
Double hybridization performed with Rick_B1, together with the universal probe EUB338, showed the absence of any other kind of endosymbiotic bacteria (Fig. 2), with the exception of gut bacteria inside adult and larval specimens of Deronectes. FISH accomplished with EUB388 II and EUB338 III did not show any positive signals (data not shown).

Electron microscopy of the Rickettsia symbiont

Ultrastructural examinations of male accessory glands of D. platynotus revealed coccobacillary rickettsiae that were observed free in the cytoplasm (Fig. 3a). Most rickettsiae ranged from 0.35 μm in width to 0.65 μm in length. Rickettsiae were delineated by an inner periplasmatic membrane, a periplasmatic space and a trilaminar cell wall with a thicker inner leaflet, characteristic of the genus Rickettsia. A Rickettsia-typical slime layer or an electron-translucent zone, up to 60 nm thick, surrounded the cell wall and separated the bacterium from the cytoplasm of the host cell (Fig. 3b). Isolated bacteria were also found in the musculature, enclosing the male accessory gland (Fig. 3c). Some of the symbionts were much longer and had a ‘filamentous’ appearance. They could be as long as 2.74 μm (Fig. 3d).

Phylogenetic analysis of the Rickettsia symbiont

Phylogenetic analyses on the basis of the 16S rRNA gene sequence revealed that Rickettsia sp. from Deronectes belong neither to the ‘spotted fever group’ nor the ‘typhus group,’ but was placed within the ancestral group (100% bootstrap support) (Fig. 4). Clones of the endosymbiotic bacteria of D. platynotus, D. aubei and D. delarouzei were placed in the clade with R. limoniae, Rickettsia endosymbionts of Cerobasis guentheri (Psocoptera, Trogidae) and Lutzomyia apache (Diptera, Psychodinae), whereas Rickettsia sp. of D. semirufus cluster basal with rickettsiae from leeches. By phylogenetic analysis based on the gltA gene (Fig. 5), the Rickettsia strain from Deronectes clustered with Rickettsia endosymbionts of L. apache and different spider species in a terminal group (99% bootstrap support) that is distinctly separated from other Rickettsia species of the spotted fever, typhus and bellii groups. The phylogenetic position of Deronectes rickettsiae was the same in the 16S rRNA gene and gltA trees when the maximum-likelihood, parsimony and distance methods were used (data not shown). Only the position of the Rickettsia endosymbiont of D. semirufus varied with different methods (MrBayes, data not shown), and was sometimes added to the leech group or to the other rickettsial endosymbionts of the Deronectes species of the limoniae group. This explains the low bootstrap support values in the ancestral group in the phylogenetic tree of 16S rRNA gene.

Discussion

The present study reports the first molecular identification of Rickettsia in four water beetle species of the genus Deronectes and generally for adephagan beetles. Evaluable results have been obtained of D. platynotus and D. aubei. Rickettsia infection was detected in 100% (45/45) of D. platynotus and 39.4% (28/71) of D. aubei. The frequencies of Rickettsia infection were maintained constant in different seasons. Individuals of D. delarouzei and D. semirufus were also tested positive for rickettsiae, whereas individuals of D. latus, D. aubei sanfilippoi and D. moestus inconspectus did not show any signal for symbiotic bacteria. Remarkably, Rickettsia-positive species of Deronectes do not cluster in a single group within the phylogenetic tree of Deronectes. In addition, the current analysis indicated six more species of Agabus and Hydropterus (Dytiscidae) that are
associated with Rickettsia sp. too. To our knowledge, only six verifications of Rickettsia have been documented in Coleoptera so far (Coccinellidae: Werren et al., 1994; von der Schulenburg et al., 2001; Bruchidae: Fukatsu et al., 2000; Buprestidae: Lawson et al., 2001; Curculionidae: Zchori-Fein et al., 2006; and Mordellidae: Duron et al., 2008). All rickettsial isolates from beetles, with the exception of Mordellistena sp., Adalia bipunctata and Adalia decempunctata, have been allocated to the rickettsial ancestral group, which includes all members of a clade that are most basal in the genus Rickettsia. Interestingly, the ancestral group comprised various symbionts from aquatic hosts, such as leeches (Kikuchi et al., 2002; Kikuchi & Fukatsu, 2005), amphizoic amoeba N. patterni (Dykova et al., 2003) and marine ciliate D. appendiculata (Vannini et al., 2005). Even insects that spend most of their development in water, i.e. the cranefly L. chorea (Diptera, Limoniidae; AF322443), mothfly L. apache (Diptera, Psychidae; EU223247) and biting midge Culicoides sonorensis (Diptera, Ceratopogonidae) (Campbell et al., 2004), have been shown to be infected with rickettsiae. The exact classification of these rickettsial isolates that are allocated to the ancestral group is still unclear. Only 16S rRNA gene sequences are available for most members of the ancestral group. However, for detailed phylogenetic analysis within the genus Rickettsia, 16S rRNA gene sequences are not convenient because of their conservatism (Roux et al., 1997). Nevertheless, rickettsial 16S rRNA clone sequences from D. platynotus are different in two to six positions from each other, mainly in two variable regions. In all sequenced clones, the sequence similarity ranges from 99.1% to 100%. Possible reasons for the microdiversity of rickettsial 16S rRNA gene clones in D. platynotus may be the presence of multiple different copies of the 16S rRNA gene in one genome, which has been demonstrated for numerous species of bacteria (Gil et al., 1996).
Fig. 4. Phylogenetic position of different clones of the endosymbiotic bacteria of water beetles Deronectes platynotus, Deronectes aubei, Deronectes semirufus and Deronectes delarouzei (bold) based on 55 16S rRNA gene sequences (1350 bp, maximum parsimony heuristic search, 50% majority-rule consensus tree of 840 most parsimonious trees, tree length = 497, CI = 0.71, RI = 0.86). The tree has been rooted with Orientia tsutsugamushi as an outgroup. Only bootstrap values of at least 50% are shown at nodes (n = 500).
Fig. 5. Phylogenetic position of the endosymbiotic bacteria of water beetles *Deronectes platynotus*, *Deronectes aubei*, *Deronectes semirufus* and *Deronectes delarouzei* (bold) based on 46 *gltA* gene sequences (275 bp, maximum parsimony heuristic search, 50% majority-rule consensus tree of 30 most parsimonious trees, tree length = 459, CI = 0.55, RI = 0.88). The tree has been rooted with *Rhodopirellula baltica* as an outgroup. Only bootstrap values of at least 50% are shown at nodes (n = 500).
Referred to rickettsiae of the ancestral group, the citrate synthase-coding gltA gene sequences, also used for phylogenetic analysis, are only available for *Rickettsia* sp. of *L. apache* (EU368001) and rickettsial isolates from spiders (Goodacre et al., 2006). Sequences of further protein-coding genes, mostly those belonging to the surface cell antigen (sca) family, which have been used for the classification of rickettsial isolates over the past few years (Fournier et al., 1998; Roux & Raoult, 2000; Sekeyova et al., 2001; Ngwami-diba et al., 2006), are not available for rickettsiae of the ancestral group (Fournier et al., 2003).

Phylogenetic analysis based on the 16S rRNA gene showed that *Rickettsia* sp. from *Deronectes* cluster into the ‘limoniae group’, the basal subgroup of the ancestral group. Furthermore, we succeeded in partial sequencing of the gltA gene of these rickettsiae. Sequences from *Deronectes* symbionts resulted in a high concordance with rickettsial isolates from spiders *P. phrygianus* and *M. mengei* (Goodacre et al., 2006). However, 16S rRNA gene sequences are not available for spider rickettsiae in GenBank. It is interesting that sequences of the gltA gene of *Rickettsia* sp. isolated from water beetles are quite divergent from all previous valid *Rickettsia* strains from the spotted fever, typhus and bellii groups. Furthermore, gltA sequences of *Rickettsia* sp. show a high similarity to the related genus *Bartonella*, whose members are known as emerging human pathogens (Minnick & Anderson, 2006), but do not cluster with *Bartonella* sequences. Goodacre et al. (2006) presumed that spider-specific *Rickettsia* form an isolated, terminal group that contains no symbionts of other arthropod hosts. They further assumed that frequent horizontal transmission of rickettsiae between spiders and their insect prey is unlikely because this would produce a heterogeneous assortment of non-spider-specific *Rickettsia* in spiders. In contrast, we were able to connect molecular data of the 16S rRNA gene sequences from basal rickettsiae strains and spider-specific *Rickettsia* by sequencing of 16S rRNA and gltA genes of the *Deronectes* symbiont. We assume that other *Rickettsia* strains of the ancestral group could exhibit comparable gltA sequences. Based on this assumption, *Rickettsia* from water beetles and spiders could belong to a new taxon, which might represent a near relative of the genus *Rickettsia*. It can be speculated that sequences of the ompA, ompB genes and gene D will show appreciable deviations like gltA, if these genes are present in rickettsiae of the ancestral group in general.

However, ultrastructural observations revealed typical morphological features and intracellular arrangements of rickettsiae, such as a thickened inner leaflet or electron-lucent ‘halos’ (Silverman, 1991). Additionally, some rickettsiae had a ‘filamentous’ appearance, similar to *R. bellii* from *Amblyomma cooperi* ticks (Labruna et al., 2004). The actual length of these bacteria cells could not be revealed by transmission electron microscopical sections.

Furthermore, *Rickettsia* could be detected in all tissues of all investigated *Rickettsia*-positive *Deronectes* species. Presumably, symbionts have been transported by the hemolymph, comparable to observations in aphids (Chen et al., 1996). This would support their appearance in the eyes and legs. However, there is no evidence of harboring of *Rickettsia* in specific sheath cells, secondary mycetocytes or mycetome-like structures in aphids or booklice (Sakurai et al., 2005; Perotti et al., 2006). Likewise, *Rickettsia* could not be detected in high densities close to midgut epithelia or malpighian tubules (Perotti et al., 2006). In contrast, the internal genitals and fatbody exhibited numerous symbionts. Rickettsiae depend on precursors of amino acids from their host. Equally, the *de novo* synthesis of purines as well as pyrimidine is not achieved by rickettsiae (Renesto et al., 2005). Hence, tissue of high metabolic activity, such as the fatbody, could be preferably infested with *Rickettsia*. The presence of bacteria within ovariols and eggs also indicates strong evidence for transovarial transmission, which must contribute to the maintenance of *Rickettsia* sp. infection in *Deronectes* populations (Whitworth et al., 2003). This is supported by the existence of *Rickettsia* sp. in the second and third larval stages of *Deronectes*, whereas the quantity of symbionts increased gradually in each stage.

Thus, the amount of bacteria seems to be determined by several factors: (1) fidelity of vertical transmission, (2) frequency of horizontal transmission (Fukatsu et al., 2000), (3) fitness effect on the host and (4) selfish manipulation of the host reproduction (Tsuchida et al., 2002). Moreover, interactions between the endosymbionts and their hosts are complicated due to subpopulation structures and fluctuating environmental conditions. The resulting bottleneck during vertical transmission could be a reason for the fluctuated rate of rickettsial affection in *D. aubei*. More individuals of *D. delarouzei* and *D. semirufus* have to be investigated to verify the infection rates. Because of the predatory nature of *Deronectes* (Dettner et al., 1986), a horizontal transmission of *Rickettsia* sp. seems to be possible. Current research should demonstrate whether aquatic prey of *Deronectes* is also associated with *Rickettsia* sp.

On the basis of the present results, there are no indications that *Rickettsia* sp. affection has an effect on the fitness of *Deronectes*. Neither reduced body weight and fecundity like in *Rickettsia*-infected host aphids (Sakurai et al., 2005) nor remarkable increases in host size as observed in leeches (Kikuchi & Fukatsu, 2005) could be observed. However, the research of fitness effects is quite difficult, because *Deronectes* cannot be bred under laboratory conditions. Parasitic living bacteria, such as *Rickettsia*, *Spiroplasma*, *Cardinium* and especially *Wolbachia*, have a tendency to manipulate host reproduction for their own benefit. Reproductive phenomena such as parthenogenesis, cytoplasmatic incompatibility, feminization and male killing have been
documented (O’Neill et al., 1997). Male killing caused by Rickettsia was described until now in ladybird beetles A. bipunctata and A. decempunctata (Werren et al., 1994; von der Schulenburg et al., 2001) and buprestid beetle Brachys tessellatus (Lawson et al., 2001). Probably, rickettsiae also influence oogenesis in bark beetle Coccytodes dactyliperda (Zchori-Fein et al., 2006). Rickettsia-induced parthenogenesis was documented from eulophid parasitoid Neochrysocharis formosa (Hagimori et al., 2006). In contrast, all investigated species of Deronectes indicated a balanced sex ratio.

In this context, further research is required to investigate in detail more about the influence of Rickettsia on Deronectes species, especially at the biochemical level. Furthermore, transmission and maintenance of Rickettsia in different populations of Deronectes species must be examined. Future studies will show, whether other Dytiscidae as well as their aquatic prey are also infected by Rickettsia sp. symbiont.

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