The genus *Helicobacter* comprises more than 30 species (9). Although a few have been thoroughly studied for their impact on human and animal health (4, 5, 15, 23) as well as on research (6), in most cases, their zoonotic potential and mode of transmission remain obscure (18), especially for intestinal helicobacters (22). In the case of rodents, most studies have been conducted with laboratory mice, and studies of wild populations across large geographical areas are virtually unknown. Therefore, screening of wild commensal rodents like the house mouse, *Mus musculus*, which is known to host at least 11 intestinal helicobacters (3, 21, 22), for the occurrence and prevalence of *Helicobacter* species is considered crucial for gaining insights into their ecology and epidemiology (8).

In this study, 425 mice were trapped at 91 sites over a 6,500-km² rectangular area stretching from northeastern Germany to western Czech Republic (Fig. 1; also, see Table S1 in the supplemental material). This area is occupied by two house mouse sub-species, *Mus mus domesticus* and *Mus mus musculus* (see references 10 and 11 for their distribution). Specifically, we asked what the prevalence and the diversity of *Helicobacter* spp. in natural populations of the house mouse are and also whether coinfection with several *Helicobacter* spp. is common in wild mice.

House mice were live-trapped inside and adjacent to houses and farms from 2008 to 2010 and dissected in a field laboratory. Extreme caution was taken to avoid cross-infections and cross-contaminations (e.g., mice were housed singly until anesthetized; sterile dissection was performed a maximum of 24 h after a mouse was trapped). The colon, including feces, of each mouse was put into a sterile 30-ml screw-cap microtube containing 70% ethyl alcohol (EtOH) and stored at room temperature. DNA was isolated from fecal samples under sterile conditions at the Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, as described elsewhere (17).

Using *Helicobacter* genus-specific primers (C97, 5′-GCT ATG ACG GGT ATC C; C98, 5′-GAT TTT ACC CCT ACA CCA), part of the 16S rRNA gene was amplified (14, 19) for each mouse sample. *Escherichia coli* was used as a negative and *Helicobacter equorum* as a positive control (provided by Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic) (1). Therefore, there is 100% prevalence of *Helicobacter* infection in wild house mice in our study area. The same level of *Helicobacter* prevalence was reported for 84 samples of three wild rodent species in Sweden; however, house mice were absent in that study (12). A lower prevalence was reported in surveys of laboratory mice (Table 1); however, this might reflect the eradication efforts adopted in most rodent vivaria against these bacteria, improvement in nutrition, or lack of continuous reinfection.

Multiplex species-specific PCR (6) was used to screen for 5 *Helicobacter* species (*Helicobacter hepaticus*, *H. bilis*, *H. rodentium*, *H. typhlonius*, and *H. muridarum*), and 401 samples were positive for at least one of these species. PCR products of the 16S rRNA from two mice each hosting only *H. hepaticus*, *H. bilis*, *H. rodentium*, *H. typhlonius*, and *H. muridarum* were sequenced (primers C97 and C98; see the sequencing method below) and found to be identical with previously published GenBank sequences (accession numbers are given in Fig. 2). No house mouse carrying only *H. muridarum* in a single infection was found; consequently, the PCR product for this species could not be sequenced. The prevalence of individual *Helicobacter* species in mouse samples was unequal, ranging from 78% for *H. rodentium* to 1% for *H. muridarum* (Table 1). *H. rodentium* was the exclusive helicobacter in 25% of 401 positive samples, whereas *H. typhlonius*, *H. hepaticus*, and *H. bilis* were present as a single species only in 14%, 6%, and 2% of 401 *Helicobacter*-positive mice, respectively. None of the *Helicobacter* species showed a specific pattern of spatial distribution; instead, the four most common *Helicobacter* species were found in mice from across the whole study area (Fig. 1).

These results suggest that coinfection of *Helicobacter* species is common in wild house mouse individuals across our large study area. Among the 401 *Helicobacter*-positive feces samples, 279 were positive for more than one species (the median was 2 *Helicobacter* species per mouse). Double infections were detected in 42% of mice, single infections were detected in 30%, and coinfections with three or four *Helicobacter* species were detected in 21% and 6% of mice, respectively. The most frequently observed helicobacter communities in double infections were *H. rodentium*/*H. typhlonius*.
and *H. rodentium*/*H. hepaticus*, each reaching 15% of the total sample of 401 mice, whereas in triple infections, the highest frequency was observed for *H. rodentium*/*H. typhlonius*/*H. bilis* (9% of mice). This is in contrast to previous reports showing that in laboratory mice, infections with single *Helicobacter* species are most commonly observed (16, 20). Further screening should confirm whether multiple infections are the prevailing pattern in wild house mice.

To identify the *Helicobacter* species in the remaining 24 samples with a negative species-specific PCR, products from positive genus-specific PCR (422-bp fragments of the 16S rRNA gene) (19) were sequenced using a BigDye Terminator v3.1 cycle sequencing kit and an ABI Prism 3130 genetic analyzer (Applied Biosystems, Carlsbad, CA). The sequences were edited in Seqscape v2.5 (Applied Biosystems), cut to 364 bp, aligned using the same software, and searched using BLAST in GenBank (accessed at http://blast.ncbi.nlm.nih.gov/Blast.cgi). In 13 samples, the presence of ambiguous bases suggested coinfection by two or more *Helicobacter* species, and these samples were excluded from further analyses. For the remaining 11 samples, a DNA-barcoding approach was

### TABLE 1 Helicobacter prevalence in laboratory and wild mice

<table>
<thead>
<tr>
<th>Helicobacter species</th>
<th>Wild house mice (this study)</th>
<th>Mice in vicinity of laboratory facilities</th>
<th>Laboratory mice</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. bilis</em></td>
<td>30</td>
<td>NA</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td><em>H. hepaticus</em></td>
<td>41</td>
<td>59</td>
<td>11–59</td>
<td>7, 16, 20</td>
</tr>
<tr>
<td><em>H. muridarum</em></td>
<td>1</td>
<td>NA</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td><em>H. rodentium</em></td>
<td>78</td>
<td>10</td>
<td>6–17</td>
<td>7, 16, 20</td>
</tr>
<tr>
<td><em>H. typhlonius</em></td>
<td>53</td>
<td>29</td>
<td>26</td>
<td>16, 20</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>8</td>
<td>11</td>
<td>7, 16</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>93</td>
<td>34–87.5</td>
<td>2, 7, 13, 16, 20</td>
</tr>
</tbody>
</table>

Values were estimated in this and previous studies. Records retrieved from the literature are directly comparable to ours, since they present data based only on PCR amplification methods. NA, not analyzed.
used, and their position on a neighbor-joining (NJ) tree was used to infer species (Fig. 2). The 16S rRNA sequences were added to GenBank: three samples appeared to represent *H. typhlonius* and one sample *H. hepaticus*; four were identical to *H. mastomyrinus*, and two clustered with *H. gannani* (all showed ≥ 99% identity in BLAST searches). The species status of the *Helicobacter* strain from mouse sample SK1050 (accession number JX198317) was not resolved (Fig. 2).

To conclude, there is a wide spectrum of *Helicobacter* species in wild house mice in central Europe. The diversity of *Helicobacter* species found in this sample of wild mice is striking: we found representatives of 7 of 11 recognized *Helicobacter* species previously identified in *Mus musculus* (3, 21, 22), plus one unknown species, in an area of only 6,500 km². Although other studies have also reported a high prevalence of helicobacters in house mice (Table 1), this study showed that all mice from all 91 sites were infected with at least one *Helicobacter* species in our study area, which included parts of two countries (Germany and Czech Republic) and two house mouse subspecies. Collectively, the results indicate that *Helicobacter* spp. could be common bacteria that infect the majority of wild house mice, but this needs to be confirmed in other geographical areas. The high prevalence of *H. bilis*, *H. hepaticus*, and *H. typhlonius* (Table 1) is intriguing given their ability to cause disease (including inflammatory bowel disease) in some strains of laboratory mice (8, 22), and these species should be studied further for their impact on house mouse ecology. Given that several of the *Helicobacter* species detected in these samples can also infect humans (*H. bilis* and *H. hepaticus*) (6, 15, 22) and domestic animals such as dogs (*H. bilis*) (22), and as house mice are mainly commensal (living in human habitations), further studies should be carefully conducted to confirm whether wild house mice could serve as a vector of *Helicobacter* infection (15). Furthermore, since several species harbored by wild mice are known to confound or potentially confound studies in laboratory mice by causing disease and are difficult to eradicate (e.g., *H. hepaticus, H. bilis, H. bilis/H. rodentium*, and *H. typhlonius* [22]), care should be taken to avoid contact of breeding colonies of house mice with their wild counterparts. Our results also indicate that wild mice could be source of novel intestinal helicobacters, and we urge further screening of wild populations.

**Nucleotide sequence accession numbers.** The 16S rRNA sequences determined here were added to GenBank: *H. typhlonius* (three samples), accession number JX198315; *H. hepaticus* (one sample), accession number JX198316; *H. mastomyrinus* (four samples), accession number JX198314; and *H. gannani* (two samples), accession number JX198318. The species status of the *Helicobacter* strain from mouse sample SK1050 (accession number JX198317) was not resolved.

**ACKNOWLEDGMENTS.** We thank many of our colleagues for collecting mouse samples. We are grateful to Bohumil Sak and Martin Kvač, Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic for providing DNA samples and to Barbora Bezděková and Ján Futas, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic for providing *H. equorum* DNA. Ludovít Dureje constructed maps of *Helicobacter* distribution. Karolína Sobeková and Tomáš Albrecht helped in initial phases of this project. Oldřich Tomášek provided valuable comments on an earlier version of the manuscript.

This work was supported by the Czech Science Foundation (project 206/08/0640) and the Fondazione Edmund Mach (HCH).

**REFERENCES.**