Description of *Candidatus* Bartonella fadhilae n. sp. and *Candidatus* Bartonella sanaae n. sp. (*Bartonellaceae*) from *Dipodillus dasyurus* and *Sekeetamys calurus* (*Gerbillinae) from the Sinai Massif (Egypt)

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Abstract

Bartonella spp. are parasites of mammalian erythrocytes and endothelial cells, transmitted by blood-feeding arthropod ectoparasites. Different species of rodents may constitute the main hosts of *Bartonella*, including several zoonotic species of *Bartonella*. The aim of this study was to identify and compare *Bartonella* species and genotypes isolated from rodent hosts from the South Sinai, Egypt. Prevalence of *Bartonella* infection was assessed in rodents (837 *Acomys dimidiatus*, 73 *Acomys russatus*, 111 *Dipodillus dasyurus*, and 65 *Sekeetamys calurus)* trapped in 2000, 2004, 2008, and 2012 in four dry montane wadis around St. Katherine town in the Sinai Mountains. Total DNA was extracted from blood samples, and PCR amplification and sequencing of the *Bartonella*-specific 860-bp gene fragment of *rpoB* and the 810-bp gene fragment of *gltA* were used for molecular and phylogenetic analyses. The overall prevalence of *Bartonella* in rodents was 7.2%. Prevalence differed between host species, being 30.6%, 10.8%, 9.6%, and 3.6% in *D. dasyurus*, *S. calurus*, *A. russatus*, and *A. dimidiatus*, respectively. The phylogenetic analyses of six samples of *Bartonella* (five from *D. dasyurus* and one from *S. calurus*) based on a fragment of the *rpoB* gene, revealed the existence of two distinct genetic groups (with 95–96% reciprocal sequence identity), clustering with several unidentified isolates obtained earlier from the same rodent species, and distant from species that have already been described (90–92% of sequence identity to the closest match from the GenBank reference database). Thus, molecular and phylogenetic analyses led to the description of two species: *Candidatus* Bartonella fadhilae n. sp. and *Candidatus* Bartonella sanaae n. sp. The identification of their vectors and the medical significance of these species need further investigation.

Keywords: *Acomys, Bartonella, Dipodillus dasyurus,* genotypes, rodent, *Sekeetamys calurus,* Sinai

Introduction

BARTONELLA SPP. ARE gram-negative bacteria found in mammalian erythrocytes and endothelial cells and are transmitted by blood-feeding arthropod ectoparasites (Birtles 2005, Morick et al. 2011). The genus *Bartonella* belongs to the *Alphaproteobacteria* class, order *Rhizobiales*, and family *Bartonellaceae* (Inoue et al. 2010). Among 33 confirmed *Bartonella* species to date, more than half are hosted by rodent species. Moreover, at least seven species have been

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linked to human diseases: *Bartonella elizabethae* (Daly et al. 1993), *Bartonella grahamii* (Birtles et al. 1995), *Bartonella rochalimae* (Lin et al. 2008), *Bartonella tamiae* (Kosoy et al. 2008), *Bartonella tribocorum* (Heller et al. 1998), *Bartonella vinsonii* (Welch et al. 1999), and *Bartonella washoensis* (Kosoy et al. 2003), most of them being associated with a wide range of symptoms, including fever, myocarditis, endocarditis, lymphadenitis, and hepatitis (Buffet et al. 2013). Certain *Bartonella* species are known to cause a febrile bacteremia in humans and other mammals, including *Bartonella quintana*, the agent of trench fever, and *Bartonella henselae*, causing a cat scratch disease (Vayssier-Taussat et al. 2016).

In almost all countries, wild rodents are infected by an extremely diverse range of *Bartonella* species/strains and generally with a high prevalence. Examples include rodents in North America (Kosoy et al. 1997), Asia (Ying et al. 2002, Inoue et al. 2008, 2010), Australia (Gundi et al. 2009), Africa (Bajer et al. 2006, Sato et al. 2013), and Europe (Birtles et al. 1995, Tea et al. 2004, Welc-Faleciak et al. 2010, Paziewska et al. 2011). Prevalence of *Bartonella* infections in rodents ranges from 6% in the Greater Jakarta area in Indonesia (Winoto et al. 2005) to 70% in the Russian Far East (Mediannikov et al. 2005, Morick et al. 2011). To date, the arthropods that have been confirmed as competent vectors for *Bartonella* are the sand fly *Lutzomyia verrucarum* as a vector for *Bartonella bacilliformis* (Noguchi and Battistini 1926) and the louse *Pediculus humanus humanus* as a vector for *B. quintana* (Swift 1920). Moreover, recent studies have revealed that a range of flea species may also play a role as vectors of *Bartonella*: the flea *Ctenophthalmus nobilis* as a vector for *B. grahamii* and *Bartonella taylorii* (Bown et al. 2004), *Ctenocephalides felis* as a vector for *B. henselae* (Chomel et al. 1996), the fleas *Megabothris turbidus*, *M. walkeri*, *Hystrichopsylla talpae*, *Ctenophthalmus assimilis*, and *Ctenophthalmus agyrtes* as vectors for *B. taylorii* (Jusko 2015), and the fleas *M. turbidus*, *M. walkeri* and *C. assimilis*, as vectors of *B. grahamii* (Jusko 2015).

Because of the difficulties in identifying *Bartonella* and distinguishing between the species through the use of conventional microscopy and biochemical tests, molecular approaches have been developed based on PCR, followed by sequencing of several housekeeping genes, such as citrate synthase (*gltA*) and RNA polymerase beta subunit (*rpoB*) genes (Norman et al. 1995, Renesto et al. 2001, La Scola et al. 2003, Inoue et al. 2008). In particular, the molecular marker genes, *rpoB* and *gltA*, have been widely used for differentiating between species because of the much lower degrees of taxonomically relevant variability in these genes within *Bartonella* species (La Scola et al. 2003). Therefore, variation in the *rpoB* and *gltA* genes can be applied as a cutoff criterion for the designation of novel species in the genus *Bartonella*.

Our long-term studies on temporal changes in the hemoparasite community (*Babesia*, *Bartonella*, *Haemobartonella*, *Hepatozoon*, and *Trypanosoma*) of rodents in Egypt (Behnke et al. 2000, Bajer et al. 2006) have led to the description of a novel species—*Babesia behnkei* (Bajer et al. 2014a), and provided molecular evidence for the existence of two different variants of *Hepatozoon* and two novel variants of *Trypanosoma acomys* in *Acomys dimidiatus* (Alsarraf et al. 2016). The aim of the current study was to identify the *Bartonella* species/strains in rodent hosts, including Wagner's gerbil *Dipodillus*

dasyurus and the bushy-tailed jird *Sekeetamys calurus*, inhabiting isolated montane valleys in the Sinai Massif, Egypt.

Materials and Methods

Field studies in Sinai, Egypt

Fieldwork was conducted over 4–5-week periods in August and September in 2000, 2004, 2008, and 2012 and was based at the Environmental Research Centre of Suez Canal University (2000, 2004) or at Fox Camp (2008, 2012) in the town of St. Katherine, South Sinai, Egypt. Trapping was carried out in four montane wadis (dry valleys) in the vicinity of St. Katherine. The local environment and general features of the four study sites (W. Arbaein, W. Gebal, W. Tlah, W. Gharaba), as well as their spatial relationships with one another, have been described previously (Behnke et al., 2000). Details of the number of rodents sampled by site and year of study are presented in Table 1. At each site, rodents were caught live in Sherman traps that had been placed among the rocks and boulders around walled gardens and occasionally along the lower slopes of wadis. These were set out at dusk and inspected in the early morning before exposure to direct sunlight. All traps were brought into the local or base camp, where the animals were removed, identified, and processed. Traps were reset the following evening.

At inspection, each of the four rodent species (*A. dimidiatus*, *Acomys russatus*, *D. dasyurus*, and *S. calurus*) was sexed, weighed, measured, and scrutinized for obvious lesions as described by Behnke et al. (2004). Ectoparasites visible during field examination were removed and placed into 70% ethanol. Blood and fecal samples were taken and animals were then either fur marked individually, or ear clipped and released close to the point of capture, or returned to the main camp at St. Katherine for autopsy. A maximum of 40% of the captured rodents from each site were culled (by agreement with the St. Katherine National Protectorate authorities).

Blood collection and DNA extraction

Thin blood smears were prepared from drops of blood taken from the retro-orbital plexus using heparinized capillary tubes of animals lightly anesthetized with ether during examination in the field and from the heart of those that were autopsied under terminal anesthesia with ether. Blood smears were air-dried, fixed in absolute methanol, and stained for 1 h in Giemsa stain in buffer at pH 7.2 or by Hemacolor stain (Merck, Germany). Each smear was examined under oil immersion ($\times 1000$ magnification). Erythrocytes infected with *Bartonella* spp. were counted in 200 fields of vision. Microscopical observation of stained blood smears was used as the only detection method for study in 2000. Thereafter, from 2004 onward, molecular techniques were also used. For these, $200\,\mu$L of whole blood was collected into 0.001 M EDTA from all the culled animals and frozen at $-20°$C. Total DNA was extracted from whole blood using the DNeasy Blood and Tissue kit (Qiagen) and stored at a temperature of $-20°$C.

Molecular characterization

For the amplification of the *rpoB* gene fragment, we used the primers 1400F 5′-CGCATTGGCTTACTTCGTATG-3′ and 2300R 5′-GTAGACTGATTAGAACGCTG-3′ (Renesto et al.

TABLE 1. PREVALENCE OF BARTONELLA SPP. BY HOST, SITE, AND YEAR

Year	Host Site	Acomys dimidiatus		Acomys russatus		Dipodillus dasyurus		Sekeetamys calurus		Total	%
		Infection	%	Infection	%	Infection	%	Infection	%		
2000	Arbaein	2/58	3.44	1/4	25	1/3	33.33	ND	ND	4/65	6.15
	Gebal	1/28	3.57	0/4	0	2/6	33.33	ND	ND	3/38	7.89
	Gharaba	1/28	3.57	0/1	0	0/2	0	ND	ND	1/31	3.22
	Tlah	0/46	0	2/6	33.33	0/2	0	ND	ND	2/54	3.70
Total		4/160	2.5	3/15	20	3/13	23.07	ND	ND	10/188	5.31
2004	Arbaein	0/43	0	0/1	0	1/4	25	ND	ND	1/48	2.08
	Gebal	10/43	23.25	1/8	12.5	15/16	93.75	ND	ND	26/67	38.80
	Gharaba	1/60	1.66	0/3	0	0/7	0	ND	ND	1/70	1.42
	Tlah	7/70	10	3/8	37.5	0/0	0	0/2	0	10/80	12.50
Total		18/216	8.33	4/20	16.66	16/27	59.25	0/2	0	38/265	14.33
2008	Arbaein	1/66	1.51	0/3	0	0/2	0	0/0	0	1/71	1.40
	Gebal	0/43	0	0/6	0	0/15	0	0/8	0	0/64	0
	Gharaba	0/52	0	0/3	0	0/2	0	0/8	0	0/65	0
	Tlah	1/80	1.25	0/8	0	0/0	0	0/6	0	1/94	1.06
Total		2/241	0.82	0/20	0	0/19	0	0/14	0	2/294	0.68
2012	Arbaein	5/64	7.81	0/0	0	3/14	21.42	0/2	0	8/80	10
	Gebal	0/46	0	0/7	0	11/22	50	2/5	40	13/80	16.25
	Gharaba	1/52	1.92	0/2	0	1/16	6.25	2/17	11.76	4/87	4.59
	Tlah	0/58	0	0/9	0	0/0	0	3/25	12	3/92	3.26
Total		6/220	2.72	0/18	0	15/52	28.84	7/49	14.28	28/339	8.25
Overall total		30/837	3.58	7/73	9.58	34/111	30.63	7/65	10.76	78/1086	7.18

2001). The PCR program was modified starting by denaturation for 5 min in 95°C, followed by 38 cycles (95°C for 45 s, 55°C for 45 s, and 72°C for 45 s), and then an extension step at 72°C for 7 min. For the *gltA* gene, we used the primers CS140f 5′-TTACTTATGATCC(GT)GG(CT)TTTA-3′ (Birtles and Raoult 1996) and CS1137r 5′-AATGCAAAAAGAACAGTAAACA-3′ (Norman et al. 1995). The PCR was started by denaturation for 2 min at 95°C, followed by 40 cycles of 1 min at 95°C, 1 min at 54°C, and 2 min at 72°C, with a final extension step of 7 min at 72°C. Reactions were performed in a 20 μL volume, containing 1×PCR buffer, 0.2 U *Taq* polymerase (Thermo Scientific), 1 μM of each primer, 2 mM dNTP (A&A Biotechnology), and 2 μL of the extracted DNA sample. Negative controls were carried out in the absence of template DNA and the positive control was genomic DNA of *B. grahamii* from our earlier study (Alsarraf 2012, Bajer et al. 2014b). PCR products were subjected to electrophoresis on a 1.5% agarose gel, stained with Midori Green stain (Nippon Genetics GmbH), and sequenced by a private company (Genomed S.A., Poland).

Genotyping and phylogenetic analysis

Analysis of 860-bp *rpoB* gene fragment. From the eight *Bartonella*-positive rodent samples (Table 2), six *Bartonella* *rpoB* sequences were obtained from *D. dasyurus* and one from *S. calurus* (Table 2). In addition, one *Bartonella* sample obtained from *A. dimidiatus* from W. Tlah was genotyped by the amplification and sequencing of a shorter 333-bp fragment of the *rpoB* gene (Paziewska et al. 2011). All obtained sequences were aligned using MEGA v. 6.0 (Tamura et al. 2013). All our consensus sequences have been deposited in the GenBank database (accession numbers KX244342, KX244343, KX244344, KX244344, and KX244346). Akaike information criterion was used in the jModel Test to identify the most appropriate model

TABLE 2. ORIGIN OF THE SAMPLES OF BARTONELLA SPP. FROM RODENTS
USED FOR GENOTYPING AND PHYLOGENETIC ANALYSIS

Gene	Sample number	Sample code	Host	Site	Year	Accession no.
rpoB	49	A	*D. dasyurus*	Arbaein	2012	KX244342
	91	B	*D. dasyurus*	Gebal	2012	KX244344
	122	C	*S. calurus*	Gebal	2012	KX244346
	59	D	*D. dasyurus*	Gebal	2004	KX244342
	104	E	*D. dasyurus*	Gebal	2004	KX244345
	224	F	*A. dimidiatus*	Tlah	2004	
gltA	49	A	*D. dasyurus*	Arbaein	2012	KX137114
	91	B	*D. dasyurus*	Gebal	2012	KX137116
	122	C	*S. calurus*	Gebal	2012	KX137118
	58	G	*D. dasyurus*	Gebal	2004	KX137115
	176	H	*D. dasyurus*	Gharaba	2012	KX137117

of nucleotide substitution. A representative tree for *rpoB* sequences was constructed using MEGA v. 6.0, applying the maximum likelihood method and a Hasegawa–Kishino–Yano (I+G) model (Fig. 2).

Analysis of 810-bp *gltA* gene fragment. The analysis of eight *Bartonella*-positive rodent samples (Table 2) yielded four *Bartonella gltA* sequences from *D. dasyurus* and one sequence from *S. calurus* (Table 2). All obtained sequences were aligned using MEGA v. 6.0 (Tamura et al. 2013). All obtained sequences of *Bartonella* spp. have been deposited in the GenBank database (accession numbers KX137114, KX137115, KX137116, KX137117, and KX137118). Akaike information criterion was used in the jModel Test to identify the most appropriate model of nucleotide substitution. A representative tree for *gltA* sequences was obtained using the maximum likelihood method and a Hasegawa–Kishino–Yano (+G) model (Fig. 3).

Statistical analyses

Prevalence (percentage of animals infected) was calculated based on microscopical observations and PCR results. For analysis of prevalence, we used maximum likelihood techniques based on log-linear analysis of contingency tables in the software package IBM SPSS (version 21.0.; IBM Corp). Initially, a full factorial model was fitted, incorporating as factors host species (four levels), year of study (four levels, each of the four surveys), and site (four levels, the four wadis). The presence of *Bartonella* was implemented as ''infection'' and was considered as a binary factor (two levels, present or absent).

Ethical issue

Rodents from St. Katherine National Protectorate were sampled by agreement with the St. Katherine National Pro-

tectorate authorities obtained for each phase of the fieldwork. A maximum of 40% of the captured rodents from each site were culled by agreement with the St. Katherine National Protectorate authorities.

Results

Family *Bartonellaceae*, Gieszczykiewicz 1939 (Vos et al. 2009)

Genus *Bartonella* Strong et al. 1915 (Vos et al. 2009)

Candidatus Bartonella fadhilae n. sp.

Type-hosts: Wagner's gerbil, *D. dasyurus* (Rodentia, Muridae, Gerbillinae) and bushy-tailed jird, *S. calurus* (Rodentia, Muridae, Gerbillinae).

Type-locality: Wadi Gebal in the Sinai Mountains, Egypt.

Type-specimens: I.91 from *D. dasyurus*, sampled on August 21, 2012, in W. Gebal, Sinai Mountains, Egypt, and I.122 from *S. calurus* sampled on August 23, 2012, in W. Gebal, Sinai Mountains, Egypt.

Vector: Currently unknown.

Representative sequences: GenBank KX244344 and KX244346 (*rpoB* gene); KX137116 and KX137118 (*gltA* gene).

ZooBank registration: *Candidatus* Bartonella fadhilae n. sp. act:A9459D34-9962-4DA6-B82B-B748277602CF

Candidatus Bartonella sanaae n. sp.

Type-host: Wagner's gerbil, *D. dasyurus* (Rodentia, Muridae, Gerbillinae)

Type-locality: Wadi Gebal in the Sinai Mountains, Egypt.

Other localities: Wadi Arbaein in Sinai Mountains, Egypt.

Type-specimens: I.59 from *D. dasyurus*, sampled on August 21, 2004, in W. Gebal, Sinai Mountains, Egypt, and I.49 from *D. dasyurus*, sampled on August 18, 2012, in W. Arbaein, Sinai Mountains, Egypt.

Vector: Currently unknown.

Representative sequences: GenBank KX244343, KX244342 (*rpoB* gene), KX137114, KX137115 (*gltA* gene).
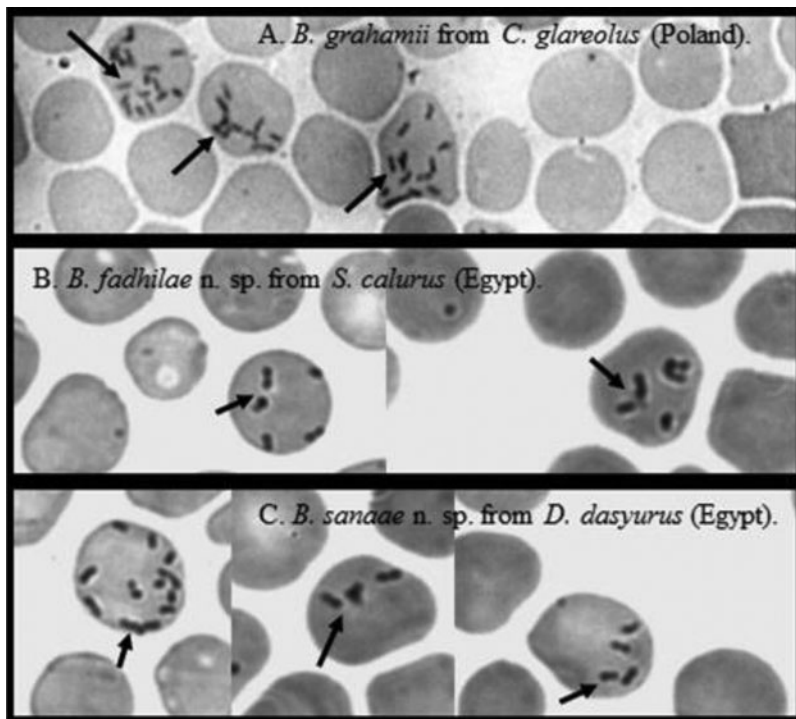
FIG. 1. **(A)** The type-forms of *Bartonella grahamii* from bank vole *Clethrionomys glareolus* from NE of Poland. **(B)** The type-forms of *Candidatus* Bartonella fadhilae n. sp. from bushy-tailed jird *Sekeetamys calurus* from W. Gebal, Sinai Massif (Egypt). **(C)** The type-forms *Candidatus* Bartonella sanaae n. sp. from Wagner's gerbil *Dipodillus dasyurus* from W. Arbaein, Sinai Massif (Egypt).

ZooBank registration: act:76DC10B7-9536-4600-930A-2920DD483C3F

Morphological characteristics

Microscopic examination of blood smears revealed that *Candidatus* Bartonella fadhilae n. sp., *Candidatus* Bartonella sanaae n. sp., and other samples of *Bartonella* sp. were indistinguishable morphologically as viewed through conventional microscopy (Fig. 1). Individual bacteria appeared as thin, short, pleomorphic bacilli or coccobacilli and were also morphologically indistinguishable from other *Bartonella* spp., known to occur in rodent hosts (Fig. 1).

Field studies: spatiotemporal dynamics
of Bartonella *infection in rodents*

Altogether, 1086 rodents from the Sinai Mountains, Egypt, were sampled in four montane valleys (wadis) in 2000, 2004, 2008, and 2012, including 837 individuals of the spiny mouse *A. dimidiatus*, 111 Wagner's gerbils *D. dasyurus*, 73 *A. russatus*, and 65 bushy-tailed jirds *S. calurus* (Table 1). Overall prevalence of *Bartonella* spp. was highest in Wagner's gerbils (30.63%) in comparison with *A. dimidiatus* (3.58%) or *A. russatus* (9.58%) and *S. calurus* (10.76%) (host species × *Bartonella* infection: $\chi^2 = 59.3$, df = 3, $p < 0.001$) (Table 1). The highest prevalence of *Bartonella* infection in the rodent communities from the Sinai was recorded in 2004—14.33% (year of study × *Bartonella* infection: $\chi^2 = 47.221$, $df = 3$, $p < 0.001$). Infections with *Bartonella* spp. were identified in all host populations and in all study sites, but we noted the highest prevalence in W. Gebal in three surveys 2000, 2008, and 2012 and the lowest was in W. Gharaba in 2000, 2004, and 2008 (year of study × site × *Bartonella* infection: $\chi^2 = 28.1$, df = 9, $p = 0.001$) (Table 1).

Molecular identification of Bartonella *sp.*
from A. dimidiatus, D. dasyurus, *and* S. calurus

We amplified the DNA of *Bartonella* spp. from eight rodent samples, six were from *D. dasyurus*, one from *S. calurus*, and one from *A. dimidiatus* (Table 2). Good quality sequences of both molecular markers *rpoB* and *gltA* were obtained only for three rodents: samples A and B from *D. dasyurus* and sample C from *S. calurus* (Table 2). *Bartonella* samples from the remaining rodents (D–H) were genotyped based only on a single marker (*rpoB* or *gltA*; Table 2).

Only one sample was successfully identified through a BLAST search in the GenBank database: sample F from *A. dimidiatus* was recognized based on the short fragment of the *rpoB* gene (333 bp). The BLAST revealed 99.37% identity of sample F to *B. acomydis* from *A. russatus* from Egypt (accession number AB602556) (Table 3).

Among the seven remaining *Bartonella* samples, seven different variants with varying degrees of similarity were recognized based on two markers (Tables 3 and 4).

Sequences of the *rpoB* gene from samples A and D, both from Wagner's gerbil, showed 99.61% identity to each other and varied by only three nucleotides and, therefore, constituted a single group (R1) (Table 3 and Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/vbz). This group showed the highest degree of similarity (91–92%) to *Bartonella florenciae* from the greater white-toothed shrew *Crocidura russula* from France. However, the

sequences that we obtained differed by 64–65 nucleotides from the reference sequence of *B. florenciae* (HM622143). Sequences of the *rpoB* gene of samples B, C, and E, from two *D. dasyurus* and one *S. calurus* individuals, showed a high reciprocal similarity (98.85–99.86%) and differed only by 1–11 nucleotides (Table 3 and Supplementary Fig. S1), and thus formed a separate group of *Bartonella* variants, named R2. For this group, the BLAST search revealed the highest similarity level (90–91%) to *B. florenciae*, although the sequences differed by as many as 67–77 nucleotides to the reference sequence (HM622143) (Table 3 and Supplementary Fig. S1). The similarity between those two groups of variants, R1 and R2, was 95–96% and the sequences varied between these two groups by 31–38 nucleotides.

The phylogenetic analyses of the *rpoB* sequences of our samples described in this work and the reference sequences from the GenBank database revealed the presence of three major groups (Fig. 2). In the first group our sequences clustered in two subgroups, R1 and R2, creating a polyphyletic group together with several unnamed isolates of *Bartonella* from *S. calurus* from Egypt (Inoue et al. 2009). The two subgroups of *Bartonella* sequences, R1 and R2, were clearly separated and distinct from each other, as confirmed by the significant bootstrap support (Fig. 2). Almost all of the *Bartonella* spp. in the phylogenetic tree that we derived are from rodents, and include two sequences from pathogenic species: *B. elizabethae* from humans from the United Kingdom and *B. bacilliformis*, but both are distinct from *Bartonella* spp. described by us.

As the analysis of the *gltA* sequences showed high similarity among sequences A, B, and G (98.7–99.5%) and our sequences differed only by 4–10 nucleotides (Table 4 and Supplementary Fig. S2), they constitute one group of *Bartonella* variants (G1). Interestingly, all these sequences were obtained from one host species, *D. dasyurus* from W. Gebal (Table 3). A BLAST search revealed that the G1 group showed the highest similarity (91–93%) to *B. acomydis* from *A. russatus* from Egypt, and our sequences differed by 18–23 nucleotides compared with the reference sequence of *B. acomydis* (AB602553) (Supplementary Fig. S2).

The *gltA* sequence of the sample C from *S. calurus* (Table 2) showed 95–96% identity to the sequences from the G1 group (26 nucleotide differences), and displayed also the highest identity (90.85%) with *B. acomydis* from *A. russatus* from Egypt (15 nucleotide differences in relation to the *B. acomydis* reference sequence AB602553) (Table 4 and Supplementary Fig. S2).

The *gltA* sequence of the unique sample H (from *D. dasyurus* from W. Gharaba) was quite distinct from all other *gltA* sequences, displaying a low similarity of 93–96% to our sequences, with 28–41 nucleotide differences, and 93% identity to *B. acomydis* from *A. russatus* from Egypt with 14 nucleotide differences (Table 4 and Supplementary Fig. S2).

The phylogenetic analyses of the *gltA* sequences revealed four main groups (Fig. 3). The samples belonging to the abovementioned group G1 clustered together with samples C and H, and created a polyphyletic subgroup, which is distinct from all other representatives within this group. Interestingly, all *Bartonella* spp. in this group are from rodents originating from different regions of the world (Fig. 3), while all other main groups contain a range of *Bartonella* species from different hosts and every group contains at least one pathogenic *Bartonella*, distinct from our sequences.

TABLE 3. PERCENT OF HOMOLOGY OF SEQUENCES FOR *BARTONELLA FADHILAE* N. SP. AND *BARTONELLA SANAAE* N. SP. WITH THE CLOSEST *BARTONELLA* SPP. USING THE *rpoB* GENE

	Bartonella florenciae HM622143		Bartonella grahamii AF165993		Sample A Candidatus Bartonella sanaae		Sample B Candidatus Bartonella fadhilae		Sample C Candidatus Bartonella fadhilae		Sample D Candidatus Bartonella sanaae		Sample E Candidatus Bartonella fadhilae		Sample F 224_Tlah_ A. dimidiatus	
	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.
Bartonella acomydis AB602556	764/825 92.60%	100%	704/789 89.22%	100%	718/789 91.00%	100%	704/786 89.56%	99%	708/789 89.73%	100%	719/789 91.12%	100%	707/789 89.60%	100%	317/319 99.37%	40%
B. florenciae HM622143			709/788 89.97%	100%	723/788 91.75%	100%	708/785 90.19%	99%	712/788 90.35%	100%	724/788 91.87%	100%	711/788 90.22%	100%	294/321 91.58%	40%
B. grahamii AF165993					702/788 89.08%	100%	696/785 88.66%	99%	702/788 89.08%	100%	703/788 89.21%	100%	699/788 88.70%	100%	274/322 85.09%	40%
Sample A *Candidatus* Bartonella sanaae							748/785 95.28%	99%	750/788 95.17%	100%	785/788 99.61%	100%	751/788 95.30%	100%	290/321 90.34%	41%
Sample B *Candidatus* Bartonella fadhilae									776/785 98.85%	99%	749/785 95.41%	99%	758/759 99.86%	99%	286/321 89.09%	40%
Sample C *Candidatus* Bartonella fadhilae											751/788 95.30%	100%	779/788 99.85%	100%	286/321 89.09%	40%
Sample D *Candidatus* Bartonella sanaae													752/788 95.43%	100%	290/321 90.34%	40%
Sample E *Candidatus* Bartonella fadhilae															286/321 89.09%	40%

Cov., coverage.

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TABLE 4. PERCENT OF HOMOLOGY OF SEQUENCES FOR *BARTONELLA FADHILAE* N. SP. AND *BARTONELLA SANAAE* N. SP. WITH THE CLOSEST *BARTONELLA* SPP. USING THE *GLTA* GENE

	B. acomydis AB602553		B. grahamii AY584855		Sample A Candidatus Bartonella sanaae		Sample B Candidatus Bartonella fadhilae		Sample C Candidatus Bartonella fadhilae		Sample G Candidatus Bartonella sanaae		Sample H Bartonella sp.	
	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.	Similarity	Cov.
B. florenciae HM622142	300/317 94.63%	26%	861/975 88.30%	99%	837/922 90.78%	99%	684/761 89.88%	99%	596/667 89.35%	92%	799/887 90.07%	99%	701/768 91.27%	99%
B. acomydis AB602553			293/330 88.78%	55%	299/322 92.85%	55%	215/234 91.88%	55%	149/164 90.85%	47%	243/261 93.10%	56%	192/206 93.20%	56%
B. grahamii AY584855					819/928 88.25%	99%	667/762 87.53%	99%	574/667 86.05%	92%	780/887 87.93%	99%	682/766 89.03%	99%
Sample A Candidatus Bartonella sanaae							752/762 98.68%	100%	640/666 96.09%	92%	864/868 99.53%	100%	739/768 96.22%	100%
Sample B Candidatus Bartonella fadhilae									643/669 96.11%	92%	752/762 98.68%	100%	707/735 96.19%	100%
Sample C Candidatus Bartonella fadhilae											639/666 95.94%	81%	625/666 93.84%	81%
Sample G Candidatus Bartonella sanaae													740/768 96.35%	100%

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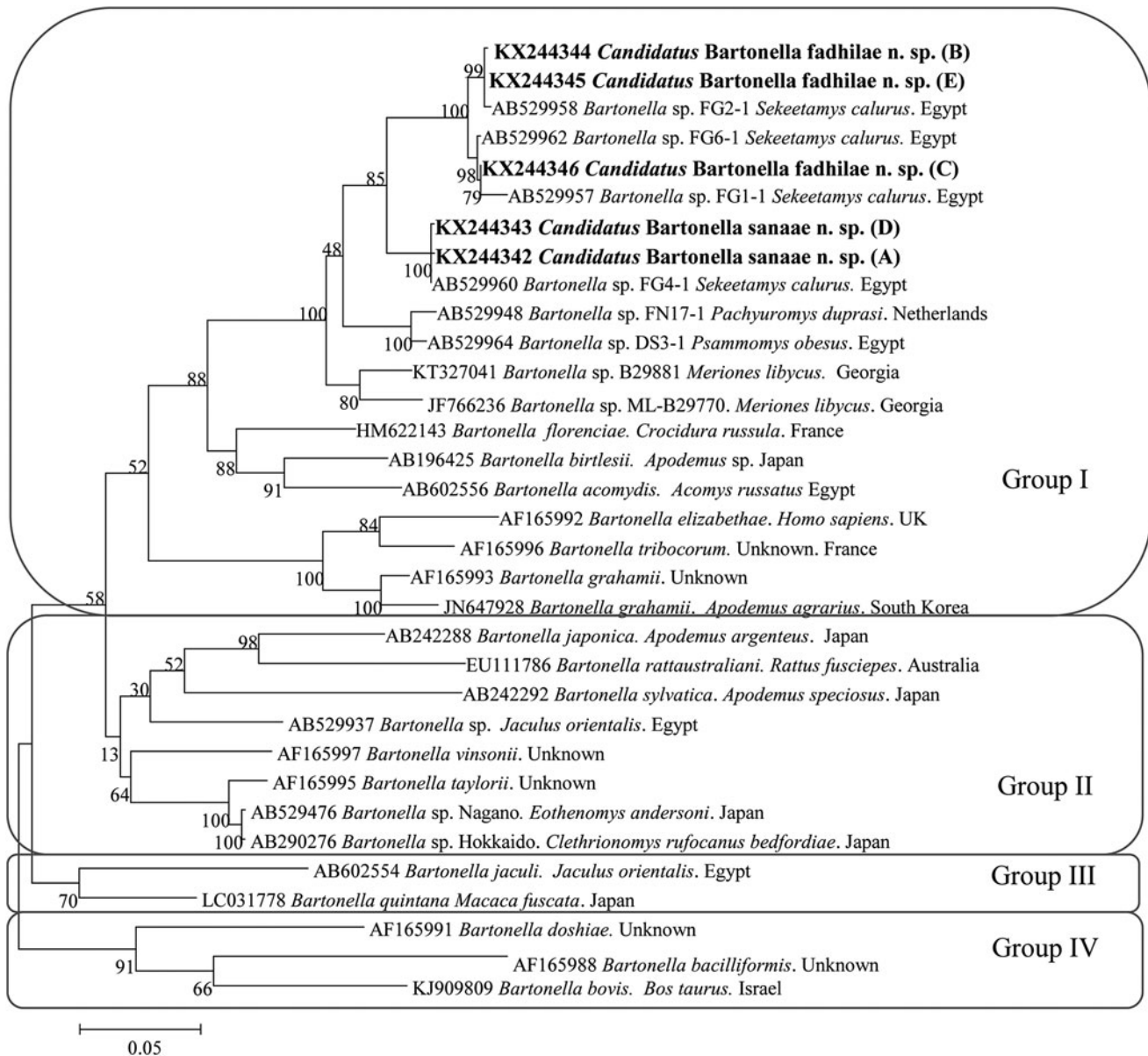
FIG. 2. The phylogenetic tree of *Bartonella* spp., based on a fragment of the *rpoB* gene, was inferred using the maximum likelihood method and a Hasegawa–Kishino–Yano (G+I). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The analysis involved 33 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6.0.

Discussion

Molecular and phylogenetic analyses of the *Bartonella* spp. infecting Wagner's gerbil and the bushy-tailed jird from the Sinai Mountains revealed their distant relationships to all known species in this genus, and therefore, two novel species of *Bartonella* are proposed. Infections with the *Candidatus* Bartonella fadhilae n. sp. were found in two host species *D. dasyurus* and *S. calurus*, and *Candidatus* Bartonella sanaae n. sp. was found only in *D. dasyurus*. Morphologically there were no differences between the two novel species of *Bartonella*.

Our study has two pertinent limitations, the principal being a lack of conventional microbiological description based on cultured isolates; none of the samples in our study was

maintained as an axenic isolate. However, our samples were collected from rodents trapped in remote sites in the deserts of the southern Sinai, and there was no possibility for initiating bacterial cultures in that setting. However, almost all known *Bartonella* species share very similar biochemical activity, as in other groups of organisms, for this genus also new species descriptions are mainly based on molecular data (Birtles et al. 1995, Gundi et al. 2004, Inoue et al. 2010, Mediannikov et al. 2013, Sato et al. 2013). Second, while the study could have benefitted from an multilocus sequence analysis, we were only able to use two genetic markers because our attempts to amplify several other genetic markers using published sets of primers were unsuccessful (16S rDNA, *ribC*, *ftsZ* (Paziewska et al. 2011) and *gro EL* (Zeaiter
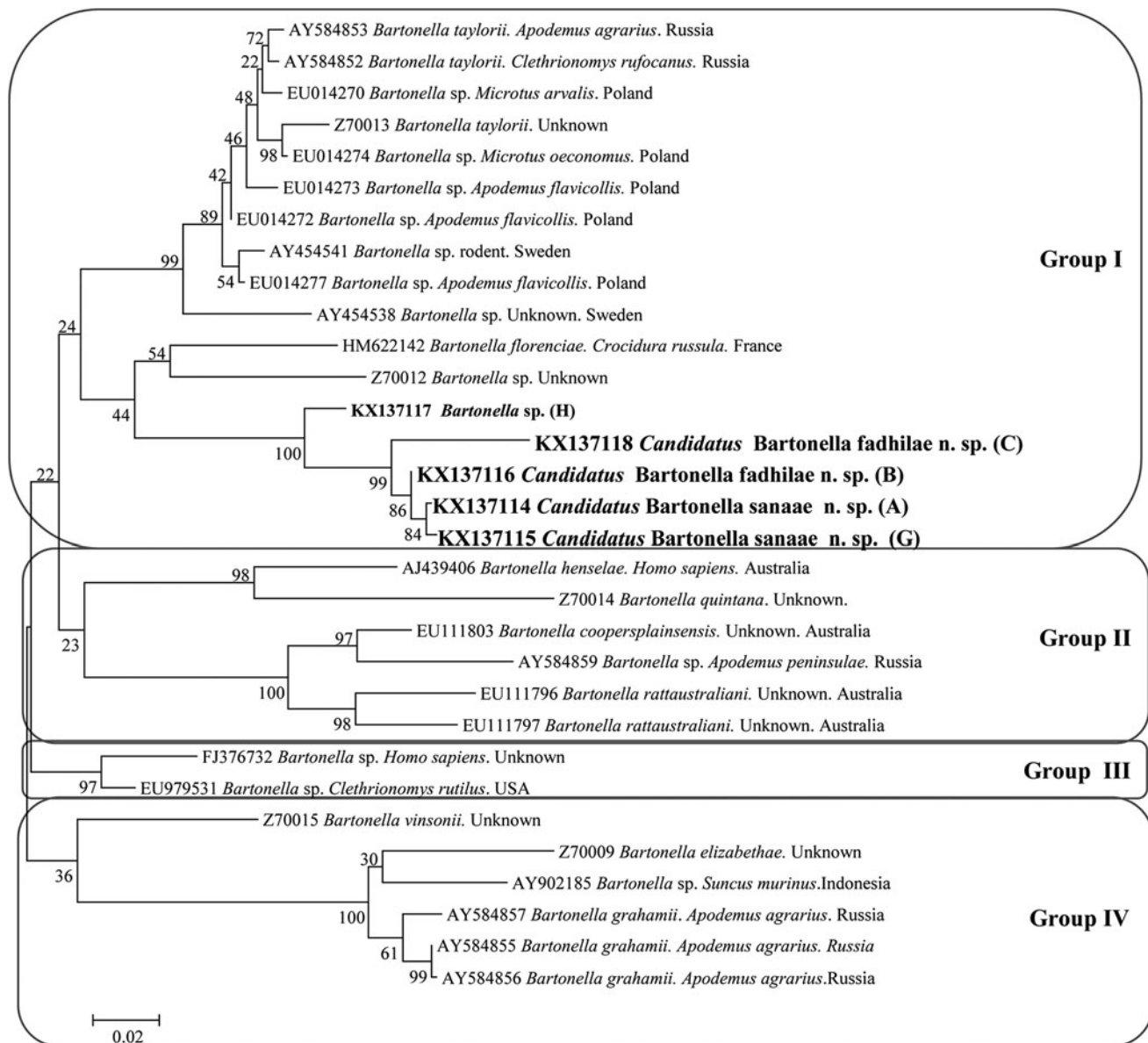
FIG. 3. The phylogenetic tree of *Bartonella* spp., based on a fragment of the *gltA* gene, was inferred using the maximum likelihood method and a Hasegawa–Kishino–Yano (G). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The analysis involved 31 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6.0.

et al. 2002). Nevertheless, our study clearly emphasizes the genetic diversification of Egyptian *Bartonella* from the species/strains of *Bartonella* described and recognized from other regions of the world.

The *rpoB* and *gltA* genetic markers enable a clear discrimination of the known *Bartonella* species (Gundi et al. 2009). Molecular analyses of the *rpoB* sequences from this and previous studies (Inoue et al. 2009) have revealed that there are two variants of *Candidatus* Bartonella fadhilae n. sp.: one infecting two host species *D. dasyurus* and *S. calurus*, and the second one that has been identified so far in only one host species *D. dasyurus* (Fig. 2). All our *rpoB* variants of *Candidatus* Bartonella fadhilae n. sp were obtained from rodents trapped in one valley, W. Gebal in 2004 and 2012,

which may suggest that it is an endemic population of *Bartonella* spp. In the case of *Candidatus* Bartonella sanaae n. sp., we also found two *rpoB* variants, both from the same host species (*D. dasyurus*) but from two isolated montane valleys, W. Gebal (2004) and W. Arbaein (2012) (Fig. 3).

It has been reported previously that the *Bartonella* isolates should be considered to represent a novel species, if the hypervariable 327 bp *gltA* gene fragment shares <96% sequence similarity and if an 825 bp *rpoB* fragment shares <95.4% sequence similarity with sequences from strains of species with validly published names (La Scola et al., 2003, Gundi et al., 2009). In the current study, we amplified and analyzed a near full-length (810 bp) fragment of the *gltA* gene with identity below 91% to *B. florenciae*, and an 860 bp long fragment of the

rpoB gene with identity below 92% to *B. florenciae*. To check, if these differences were spread throughout the whole gene fragment, we analyzed and compared also genetic diversity among 190 bp of the hypervariable 327 bp *gltA* gene fragment extracted from our longer sequences and four reference sequences of *Bartonella* (*Bartonella doshiae* AF207827, *B. grahamii* Z70016, *B. henselae* L38987, and *B. taylorii* AF191502) from the Gen-Bank database. The latter all contained the hypervariable 327 bp gltA gene fragment (La Scola et al. 2003, Gundi et al. 2009). Although the fragment that we compared was short (190 bp), sequence homology based on this region was only 90–95% (data not presented). Accordingly, these low similarity values for two genetic markers justify the description of two new species, supported also by phylogenetic analyses.

The similarity of our *rpoB* sequences with the human pathogenic *B. elizabethae* was only 88.30% in the case *Candidatus* Bartonella fadhilae n. sp. and 88.62% for *Candidatus* Bartonella sanaae n. sp. Moreover, analyses of our *gltA* sequences showed a similarity of 88.46% for *Candidatus* Bartonella fadhilae n. sp. with the pathogenic *B. henselae* and 88.80% similarity between *Candidatus* Bartonella sanaae n. sp. and *B. henselae*. Thus, any possible pathogenic consequences of infection with these new species cannot be currently assessed. Two pathogenic *Bartonella* spp. from humans in Africa are known to exist, *B. henselae* from Namibia (Noden et al. 2014) and *B. quintana* from Senegal (Diatta et al. 2014). Both these pathogenic *Bartonella* species have been reported also in South Africa with a high prevalence of up to 23% in people with HIV (Trataris-Rebisz 2012).

Our phylogenetic analyses of the *rpoB* sequences revealed four main genetic groups. In the first, the *Candidatus* Bartonella fadhilae n. sp. and *Bartonella sanaae* n. sp. sequences cluster with an unnamed *Bartonella* spp. from *S. calurus* from Egypt (Inoue et al. 2009), comprising a polyphyletic group/clade, supported by high bootstrap values, and a separate group/clade of other *Bartonella* sp. sequences from different regions of the world and derived from different hosts. This phylogenetic tree for *rpoB* sequences clearly highlights the difference between our sequences belonging to groups R1 and R2, and shows that these groups are separate from all other named *Bartonella* spp. With the exception of only one sequence of the pathogenic *B. elizabethae*, isolated from a human heart (Daly et al. 1993), all other *Bartonella* spp. sequences from this group/clade are derived from rodents.

Our phylogenetic analyses of *gltA* sequences also revealed four groups, the first comprising our *Candidatus* Bartonella fadhilae n. sp. and *Candidatus* Bartonella sanaae n. sp., with one sequence from *D. dasyurus* (sample H) from Egypt. This group clusters with *B. florenciae* from the greater white-toothed shrew *C. russula* from France and one unnamed *Bartonella* sp.; otherwise, this group contains different species of *Bartonella* from rodents from different regions of Europe.

The topology of the *gltA* tree differs from the *rpoB* tree primarily because of sample B, which belongs to group R2 of *Candidatus* Bartonella fadhilae n. sp. and is closely related to group G1 of *Candidatus* Bartonella sanaae n. sp. As reported previously by Paziewska et al. (2011), diversity in the *gltA* gene is highly associated with host specificity. Both samples in group G1 are from the same host species *D. dasyurus* and that is most likely the reason for sample B from *D. dasyurus*, identified as *Candidatus* Bartonella fadhilae n. sp, ending up so close to group G1 of *Candidatus* Bartonella sanaae n. sp. and why sample C

from *S. calurus* identified as *Candidatus* Bartonella fadhilae n. sp. is separated from group G1 on this tree. This difference between samples B and C in the *gltA* gene sequence reflects also the difference that is apparent in the *rpoB* tree between the two variants of *Candidatus* Bartonella fadhilae n. sp.

An alternative interpretation of the differences between the two phylogenetic trees may be the possibility that horizontal gene transfer has occurred in the vector (flea), as reported in Paziewska et al. (2011). There is no recognized vector specificity for *Bartonella* spp. (Jusko 2015), and therefore, a horizontal gene transfer of the *gltA* gene is theoretically possible if a flea happens to be coinfected concurrently by two different species of *Bartonella* sp. from different hosts. As all the most similar *gltA* sequences (G1) originated from only one valley (W. Gebal), the possibility of a horizontal gene transfer of this marker cannot be excluded. Unfortunately, no other *gltA* sequences from the same or related host species are available from the GenBank database for comparison, in contrast to the *rpoB* gene, for which several sequences from *S. calurus* from Egypt have been deposited by Inoue et al. (2009) and are very similar to our sequences (although the exact region of origin in Egypt is not provided—the *Bartonella* strains in this study were isolated from rodents imported to Japan from Egypt, as pets). For these reasons, we consider the results of our phylogenetic analysis based on *rpoB* sequences to be more reliable, and based on this analysis, we have classified sample B from *D. dasyurus* as *Candidatus* Bartonella fadhilae n. sp.

Conclusions

Molecular and phylogenetic analyses of *Bartonella* samples from rodents from the Sinai Massif of Egypt have led to the description of two new species: *Candidatus* Bartonella fadhilae n. sp. and *Candidatus* Bartonella sanaae n. sp. The identification of their vectors, full microbiological assessment, and the medical significance of these species require further investigation.

Acknowledgments

Authors' Contributions

A.B. designed the study and supervised laboratory and field analyses, M.A., R.W.F., and L.D. performed molecular and phylogenetic studies, M.B. participated in biological characterization of novel *Bartonella* spp., F.G. and S.Z. organized and supervised field work in Sinai, E.J.M., J.B.B., E.M.M., and J.M.B. carried out the laboratory and field

studies and contributed to the drafting of the manuscript. All authors read and approved the final version of the manuscript.

Author Disclosure Statement

No competing financial interests exist.

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