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ORIGINAL ARTICLE

Diversity of Trypanosomatids in Cockroaches and the Description of *Herpetomonas tarakana* sp. n.

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ABSTRACT

In this study, we surveyed six species of cockroaches, two synanthropic (i.e. ecologically associated with humans) and four wild, for intestinal trypanosomatid infections. Only the wild cockroach species were found to be infected, with flagellates of the genus *Herpetomonas*. Two distinct genotypes were documented, one of which was described as a new species, *Herpetomonas tarakana* sp. n. We also propose a revision of the genus *Herpetomonas* and creation of a new subfamily, Phytomonadinae, to include *Herpetomonas*, *Phytomonas*, and a newly described genus *Lafontella* n. gen. (type species *Lafontella mariadeanei* comb. n.), which can be distinguished from others by morphological and molecular traits.

FLAGELLATES of the family Trypanosomatidae are obligate parasites of vertebrates, plants, and invertebrates. Dixenous species shuttle between two hosts (an invertebrate and a vertebrate for the genera *Trypanosoma* and *Leishmania*; or an invertebrate and a plant for the genus *Phytomonas*) during their life cycle. Monoxenous trypanosomatids (i.e. parasitizing one host, usually an insect) have recently attracted more attention due to their high diversity and virtually ubiquitous presence and thus, an important role they may play in ecosystems and biomes (Maslov et al. 2013). These flagellates are also widely used by parasitologists as model systems helping to understand how their extremely successful parasitic life style has emerged and evolved (Lukeš et al. 2014). Formerly, the taxonomy of the family Trypanosomatidae was based on cell morphotypes and details of the life cycle that resulted in inconsistency among the classification and experimental data. Now the taxonomic system of these flagellates is undergoing a significant development mostly powered by molecular methods (Votýpka et al. 2015). Recently, some old taxa were redefined and new ones were characterized with the use of molecular phylogenetic approaches (Borghesan et al. 2013; Votýpka et al. 2013; Yurchenko et al. 2008). The 18S ribosomal RNA, spliced leader RNA, and glycosomal glyceraldehyde 3-phosphate dehydrogenase (gGAPDH) genes, as well as the internal

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transcribed spacers (ITS) 1 and 2 sequences are usually applied to infer phylogenetic relationships between different groups of trypanosomatids (Maslov et al. 2013; Teixeira et al. 2011; Votýpka et al. 2014).

Currently, the family Trypanosomatidae comprises three formally described subfamilies: endosymbiont-harboring Strigomonadinae, flea-inhabiting Blechomonadinae, and Leishmaniinae that brings together monoxenous genera, such as *Leptomonas* and *Crithidia*, with the dixenous genus *Leishmania* (Jirků et al. 2012; Votýpka et al. 2013, 2014). Other monophyletic taxa of Trypanosomatidae studied to date using molecular methods are the dixenous genera *Trypanosoma* and *Phytomonas* along with the monoxenous *Herpetomonas*, *Sergeia*, *Blastocrithidia*, *Paratrypanosoma*, and *Wallacemonas* (Borghesan et al. 2013; Caicedo et al. 2011; Dollet et al. 2012; Hamilton et al. 2004; Kostygov et al. 2014; Svobodová et al. 2007). These taxonomic units have still not been assigned to subfamilies creating an imbalance in the classification.

Some significant changes made in the taxonomy of trypanosomatids, namely the description of several new genera, were associated with the discovery of new lineages during biodiversity studies. Switching from the commonly inspected hosts, such as heteropteran bugs and brachyceran (dipteran) flies, to less studied insect groups lead to the establishment of new genera, as was the case of *Blechomonas* (from fleas), *Sergeia* (from a biting midge) and *Paratrypanosoma* (from a mosquito) (Flegontov et al. 2013; Svobodová et al. 2007; Votýpka et al. 2013). The flies hosting the latter two genera belong to Nematocera (thread-horns), a suborder comprising mosquitoes, crane flies, gnats, black flies, and midges.

There are numerous insect taxa previously reported to host trypanosomatids. In the majority of them parasites have not been investigated using molecular methods. One such prominent group is the globally present cockroaches (Insecta: Dictyoptera: Blattodea), considered among the most notorious co-inhabitants of human establishments including houses, food facilities, and hospitals. There are over 4,600 valid species of cockroaches recognized to date (Beccaloni and Eades 2015), with about 50 of them being domestic or peridomestic (Brenner 1995). Cockroaches are implicated in carrying and disseminating medically relevant bacteria, fungi, helminths, and protists (Fotedar et al. 1991; Kinfu and Erko 2008; Pai et al. 2003).

The presence of trypanosomatids in cockroaches was first documented about a century ago in *Blatta orientalis* (Laveran and Franchini 1920). The discovered species was named *Herpetomonas periplanetae,* but 6 yr later Wenyon placed it into the genus *Leptomonas* (Wenyon 1926). In the taxonomical records of Trypanosomatidae (Podlipaev 1990; Wallace 1966), the new combination was preserved because it could be better accommodated into the morphotype-based classification used at that time. Another species, *Leptomonas blaberae*, was described from the tropical cockroach *Blabera* sp. in South America (Tejera 1926). Since then only three case reports about trypanosomatids in Blattodea (concerning three *Parablatta* spp. and two *Periplaneta* spp.) were published and all of them came from the New World (Fernádez et al. 2014; Pacheco and Morua 1976; Semans 1939). From these scarce publications it was difficult to judge whether cockroaches can serve as specific hosts of trypanosomatids, or become just transiently infected with parasites of their prey due to their detritophagous feeding habit.

In this work, we analyzed the presence and diversity of monoxenous trypanosomatids in four wild and two synanthropic species of cockroaches captured in the Czech Republic and Slovakia and described a new species *Herpetomonas tarakana* sp. n. In addition, based on molecular phylogenetic data, we propose transferring *Herpetomonas mariadeanei* into a newly erected genus *Lafontella* and unite it with the genera *Herpetomonas* and *Phytomonas* into a new subfamily Phytomonadinae.

MATERIALS AND METHODS

Field work, establishing of cultures, and cultivation

Sixty three specimens of wild cockroaches belonging to four species—*Ectobius lapponicus* (Linnaeus, 1758), *Phyllodromica chladeki* Harz, 1977, *Ph. hungarica* Vidlička, 1993, and *Ph. maculata* (Schreber, 1781)—were collected between May and June, 2014 in four different localities in the Czech Republic and Slovakia (Table 1). *Phyllodromica chladeki* is a very rare species, endemic to central Slovakia (Bohn and Chládek 2004). In addition, 42 individuals of two synanthropic species, American cockroach *Periplaneta americana* (Linnaeus, 1758) and German cockroach *Blattella germanica* (Linnaeus, 1767) caught in Prague and Ostrava houses (Czech Republic), were analyzed in the same way (Table 1). Insects were dissected, examined under a microscope and samples from their intestines were prepared as described previously (Votýpka et al. 2014; Yurchenko et al. 2008, 2009). To establish the primary culture, content of the insect intestine was cultivated in the Brain Heart Infusion (BHI) medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10 μg/ml of hemin (Jena Bioscience GmbH, Jena, Germany), 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin (all from Life Technologies, Carlsbad, CA). Once established, cell culture was routinely passaged in the complete M199 medium (Life Technologies) or BHI supplemented as above. The obtained culture was deposited in the collections of the Life Science Research Centre of the University of Ostrava, Department of Parasitology at Charles University in Prague, and in the Institute of Parasitology, České Budějovice, Czech Republic. It is available upon request.

Light and electron microscopy

Light microscopy of Giemsa or 4′,6-diamidino-2-phenylindole (DAPI) stained smears on poly-*L*-lysine-coated slides was done as described elsewhere (Yurchenko et al. 2006b) using an Olympus BX51 microscope equipped with a DP70 CCD camera (Olympus, Tokyo, Japan). Standard measurements were performed for 50 cells on Giemsa-stained smears and expressed in micrometers. For scanning elec-

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Table 1. List of analyzed host species showing their geographic origin (if applicable), associated isolates, and prevalence of infection

Host	Collection locality	GPS	Isolates	Infection rate
	Free-living cockroaches			
Ectobius lapponicus	CZ: Šilheřovice, Černý les Nature Reserve	N49°54′16″ E18°16′28″	OSR18, 24-30	8/28
	SK: Muráň, Muránska planina National Park, Poludnica Nature Reserve	N48°45′48″ E20°01′47″	–	
Phyllodromica chladeki	SK: Muráň, Muránska planina National Park, Poludnica Nature Reserve	N48°45′48″ E20°01′47″	OSR21	1/10
Phyllodromica hungarica	SK: Muráň, Muránska planina National Park, Suché doly Nature Reserve	N48°24′42″ E18°13′21″	OSR22	1/8
Phyllodromica maculata	CZ: Štramberk, Kamenárka Nature Monument	N49°35′26″ E18°07′23″	OSR19, 20, 32-35	6/17
Total				16/63
	Synanthropic cockroaches			
Periplaneta americana	CZ: Service bldg, Charles University, Prague			0/14
	CZ: Glasshouse of IDM, Prague			0/15
Blattella germanica	CZ: Disinfection service, Ostrava			0/13
Total				0/42

tron microscopy (SEM), cultured cells were fixed in 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). Following postfixation in 2% OsO_4 in 0.1 M phosphate buffer for 2 h, dehydration in an ascending acetone series, and critical point-drying with CO_2, samples were observed using a JEOL JSM-7401-F microscope (JEOL, Tokyo, Japan). High-pressure freezing transmission electron microscopy (HPF-TEM) was performed essentially as described elsewhere (Yurchenko et al. 2014). As a comparison, the classical chemical way of fixation was also performed: after dehydration in graded series of ethanol mixtures, the cells were embedded in Epon-Araldite, thin sections were stained with lead citrate and uranyl acetate. Images were captured on JEOL JEM–2100F microscope (JEOL) using an Orius SC1000 CCD camera (Gatan, München, Germany).

PCR amplification, cloning, and sequencing

Total genomic DNA was isolated from the field samples (dissected guts of the cockroaches) and the axenically grown culture (5 ml) using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The 18S rRNA gene (~2.1 kb) was amplified using primers S762 and S763 and sequenced directly as described previously (Maslov et al. 1996; Yurchenko et al. 2006a). The gene encoding glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH, ~1.1 kb) was amplified using primer pairs M200–M201, and sequenced as above (Maslov et al. 2010). The GenBank accession numbers for the new sequences determined in this work are KR868690 (18S rRNA of OSR18), KR868691 (18S rRNA of OSR27), and KR868692 (gGAPDH of OSR18).

Phylogenetic analyses

The 18S rRNA sequences of 53 species of trypanosomatids were aligned using Muscle 3.8.3.1 (Edgar 2004), the resulting alignment was refined manually using the BioEdit sequence alignment (Hall 1999) and ambiguously aligned positions were removed as described before (Chistyakova et al. 2014). It was then concatenated with corresponding alignment of gGAPDH gene sequences (Yurchenko et al. 2006a). The resulting dataset including 3,135 (2,043 + 1,092) positions was then used for phylogenetic inference under partitioned model with maximum likelihood criterion and Bayesian approach in Treefinder v. 03.2011 and MrBayes 3.2.5 (Jobb et al. 2004; Ronquist et al. 2012). Analysis in Treefinder was performed with the following parameters: GTR + G model for 18S rRNA gene and TVM + G, GTR + G, J3 + G correspondingly for the three codon positions of gGAPDH gene (as selected by the built-in model selector of Treefinder using Akaike information criterion); five gamma categories; optimized substitution rates; nucleotide frequencies; and partition rates. Edge support was estimated by two methods: expected likelihood weights and classical bootstrap with 1,000 replicates each. Bayesian inference of phylogeny was accomplished with analysis run for 3 million generations under GTR + I + G substitution model (five gamma categories) with nucleotide frequencies, substitution rates, partition rates, and parameters of rate heterogeneity among sites unlinked for all four partitions (defined as above). Other default parameters were as follows: number of chains, 4; default temperature, 0.2; burn-in fraction, 25%. The convergence was assessed by calculating standard deviation of the split frequencies (below 0.01), and estimating state changes between chains (between 0.3 and 0.7) and the potential scale reduction factor (close to 1). Sampling was performed every 1,000 generation.

Experimental infection

Three cockroach species cultivable in the laboratory, *P. americana* (American cockroach), *Nauphoeta cinerea* (Olivier, 1789) (speckled cockroach), and *Blatella germanica* (German cockroach) were used for experimental infection with the culture of trypanosomatid isolate OSR18. A total of 14–21 uninfected specimens of each species (both males and females) were starved for 5–12 d, and then fed dry bread presoaked in a trypanosomatid culture for

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30–60 min. Prevalence and intensity of infection were analyzed on day 1, 6, and (in some cases) 13 postfeeding by checking of feces or dissection.

RESULTS

Analysis of trypanosomatid diversity in cockroaches

From 63 specimens of wild cockroaches belonging to four different species (Table 1) and analyzed for the presence of trypanosomatids, 16 were found infected. The number of cells observed in each case was low (one to several per view field). The prevalence of infection varied between host species from 10% in *P. chladeki* to 35% in *Ph. maculata*. None of the 42 specimens of two synanthropic cockroach species was positive for trypanosomatids by light microscopy or nested PCR of the 18S rRNA gene. The sampling was not equal for different cockroach species, thus no statistically sound conclusion can be made about host specificity of trypanosomatid infection.

Isolation and primary characterization of a new trypanosomatid species from cockroaches

The dusky cockroach *E. lapponicus* (Dictyoptera: Blattodea) captured in May 2014 in the vicinity of Šilheřovice, Czech Republic (N49°54′16″ E18°16′28″) was found harboring trypanosomatids. The axenic culture OSR18 was successfully established. The monospecific nature of the culture has been confirmed by amplifying and sequencing a range of molecular markers—18S rRNA, gGAPDH, and ITS1 (data not shown). Several morphotypes encountered in situ have also been detected in vitro (see below). The 18S rRNA gene sequence amplified from the culture turned out to share 100% identity to that of the corresponding cockroach gut sample (GenBank acc. no. KR868690), confirming the identity of the cultured isolate. Sequences of five positive ones—OSR21, OSR26, OSR28, OSR33, and OSR34 from *E. lapponicus*, *Ph. maculata*, and *Ph. chladeki* captured in three different localities in the Czech Republic and Slovakia (Table 1)—were also identical to that of the OSR18 culture. This suggests that they all belong to the same trypanosomatid species. However, the samples OSR27 (from *E. lapponicus* captured near Šilheřovice, Czech Republic) and OSR32 (from *Ph. maculata* collected near Štramberk, Czech Republic) contained a different species of the genus *Herpetomonas*. Interestingly, one sample, OSR29 (with same origin as OSR27) was found to host both above-mentioned species simultaneously.

Light microscopic and ultrastructural characterization

Hereafter, we prefer using the unambiguous term "endomastigotes" instead of the more commonly used term "amastigotes". The first one is to be used for cells with intracellular flagellum and the second one—for those lacking the flagellum (as in cyst-forming trypanosomatids) (Frolov 1994). The term "endomastigote" was originally proposed for describing life cycle stages of *H. mariadeanei* and *Leishmania* spp. (Rondanelli et al. 1987; Yoshida et al. 1978). Later some authors erroneously narrowed the meaning of the term only for cells with a flagellum forming a loop around the nucleus, now named brochomastigotes (Kostygov et al. 2014).

Light microscopic examination of OSR18 culture revealed several morphotypes (Fig. 1A). Importantly, the cells observed in vitro resembled those detected in situ. Most of them were promastigotes or choanomastigotes (Fig. 1A; labeled *pr* and *ch*), with some members of the latter morphotype having a significantly shortened flagellum (Fig. 1A; *sf*). Interestingly, in the cockroach intestine such choanomastigote-like cells were predominant. The relative proportions of flagellates belonging to the opistomorph and endomastigote morphotypes (Fig. 1A; labeled *op* and *en*) were low. Promastigotes ranged from 6.6 to 14 μm (9.8 ± 2.0 μm; hereafter $N = 50$) and from 1.6 to 4.0 μm (2.9 ± 0.5 μm) in length and width, respectively. An averaged ratio between length and width was 3.4 ± 0.8. The choanomastigotes were between 4.7 and 9.6 μm (6.5 ± 1.1 μm) long and 3.6–6.2 μm (4.9 ± 0.8 μm) wide, with an averaged ratio between length and width being 1.4 ± 0.2. The single flagellum varied in length from 5.2 to 15.3 μm (10.0 ± 2.3 μm) for promastigotes and from 1.5 to 12.3 μm (5.7 ± 3.1 μm) for choanomastigotes. The kinetoplast was positioned within 0.2–4.1 μm (2.1 ± 0.8 μm) in promastigotes and within 0.1–2.9 μm (1.0 ± 0.9 μm) in choanomastigotes, from the nucleus. Staining with DAPI demonstrated the absence of endosymbiotic bacteria (Fig. 1B).

Next, the OSR18 culture cells were analyzed by SEM (Fig. 2A–C) and HPF-TEM (Fig. 2D–G). The major morphotypes (promastigotes as well as choanomastigotes with normal and shortened flagella) were observed by SEM (Fig. 2A, B, C, respectively). Flagella were widened at the opening of the flagellar pocket (Fig. 2A–E) and contacted the cell body within the pocket by means of multiple desmosomes (up to 10 on transversal sections, and up to six on longitudinal ones) (Fig. 2F; white arrowhead). Similar multiple desmosomes were observed between the flagellum and the cell wall. All typical trypanosomatid traits, such as oval nucleus, basal body, glycosomes, acidocalcisomes, Golgi apparatus, and the most characteristic feature—mitochondrial DNA compactly packed in the electron-dense kinetoplast disk, were observed by HPF-TEM (Fig. 2D, E). Kinetoplast measured between 467 and 810 nm (593 ± 82 nm, hereafter $N = 45$) in length and 115–162 nm (138 ± 11 nm) in width. Importantly, when kinetoplast dimensions were measured after the chemical (not HPF) fixation, the correspondent numbers were 668 ± 123 nm (length, $N = 45$) and 116 ± 14 nm (width), underlying the importance of comparing measurements obtained in the same way. All cells had a well-developed contractile vacuole complex located in the anterior part (Fig. 2E). Although the cytopharyngeal complex was reduced, the corresponding groups of microtubules were present (Fig. 2G, H; white arrows). In choanomastigotes, two arrays of microtubules composed of five and six each

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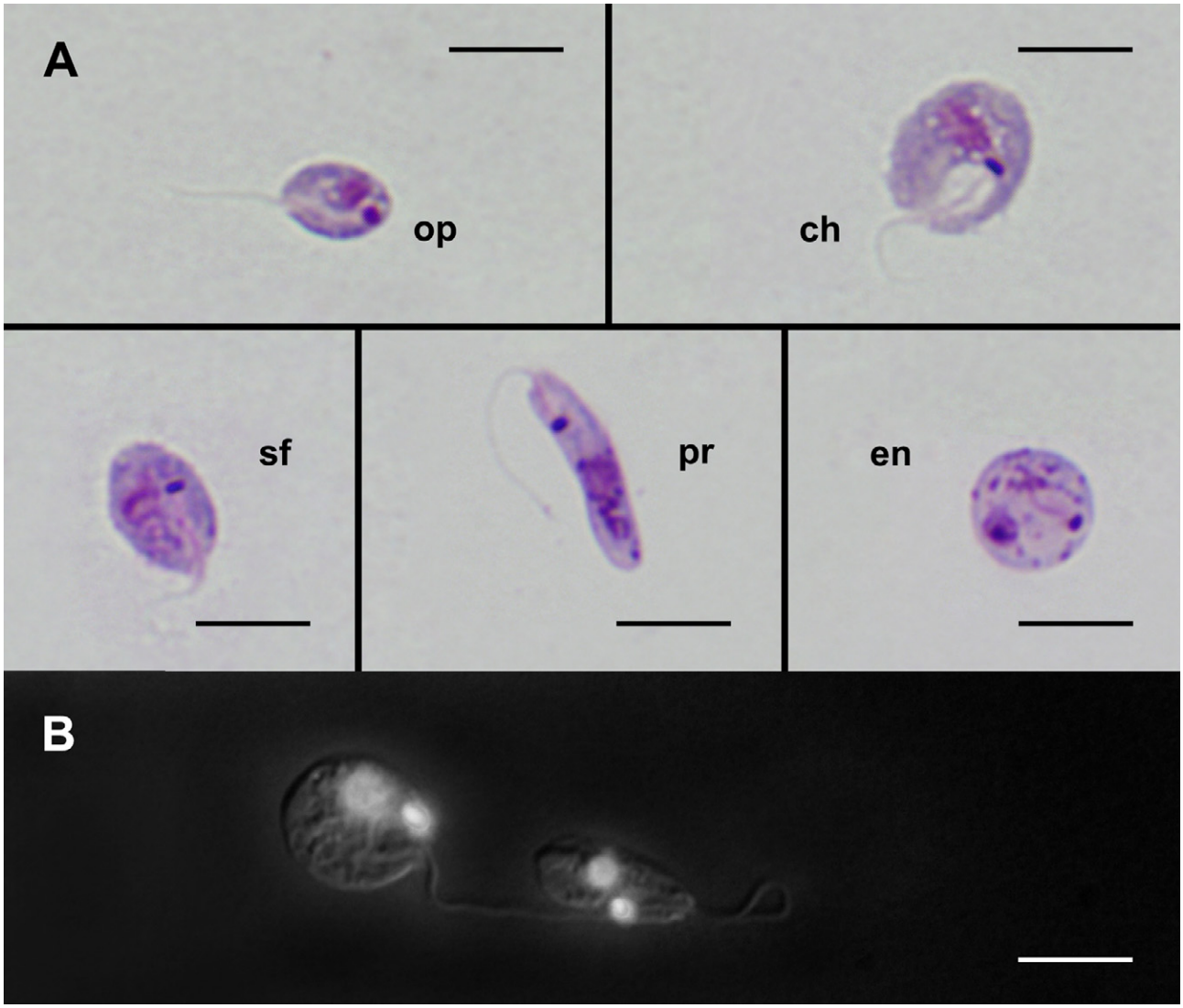

Figure 1 Light microscopy of *Herpetomonas tarakana* (isolate OSR18). **A**. Cell morphotypes detected by Giemsa staining: opistomorphs (op), choanomastigotes with various relative positions of kinetoplast and nucleus (*ch*), cells with shortened flagellum (*sf*), promastigotes (*pr*), and endomastigotes (*en*) are shown. **B**. DAPI-stained *H. tarakana* cells analyzed by the differential interference contrast combined with the fluorescent microscopy revealed the same features. Scale bars are 5 μm.

supported the characteristic cleft-like invagination of the bottom of the flagellar pocket (Fig. 2D; black arrowhead). Transversal sections through the central region of the cell demonstrated ridged nature of the cell surface, which was prominently visible in the SEM photos (Fig. 2A, B, G). These ridges often contained mitochondrial branches (Fig. 2G, labeled m). In contrast to *Kentomonas sorsogonicus* (Votýpka et al. 2014), these branches never came close to the pellicle and the layer of subpellicular microtubules was never disrupted.

Thermoresistance

As it is known that some monoxenous trypanosomatids including species of the genus *Herpetomonas* can survive elevated temperatures, we attempted growing the OSR18 culture at 35 °C for 7 d. The cultured isolate was able to withstand this temperature and multiplied normally (data not shown). Interestingly, the ratio between promastigotes and choanomastigotes changed dramatically from 93:7 (N = 144) at 25 °C to 17:83 (N = 140) at 35 °C.

Phylogenetic analyses

The 18S rRNA gene of OSR18 showed 97% identity with the sequences of *Herpetomonas nabiculae* (isolates B08-873 and Nfm2, GenBank acc. nos KF054113 and JN624300, respectively) and *H. samueli* (isolate TCC003E, GenBank acc. no. JQ359722) (Kostygov et al. 2011). In addition, two environmental isolates (Trypanosomatidae

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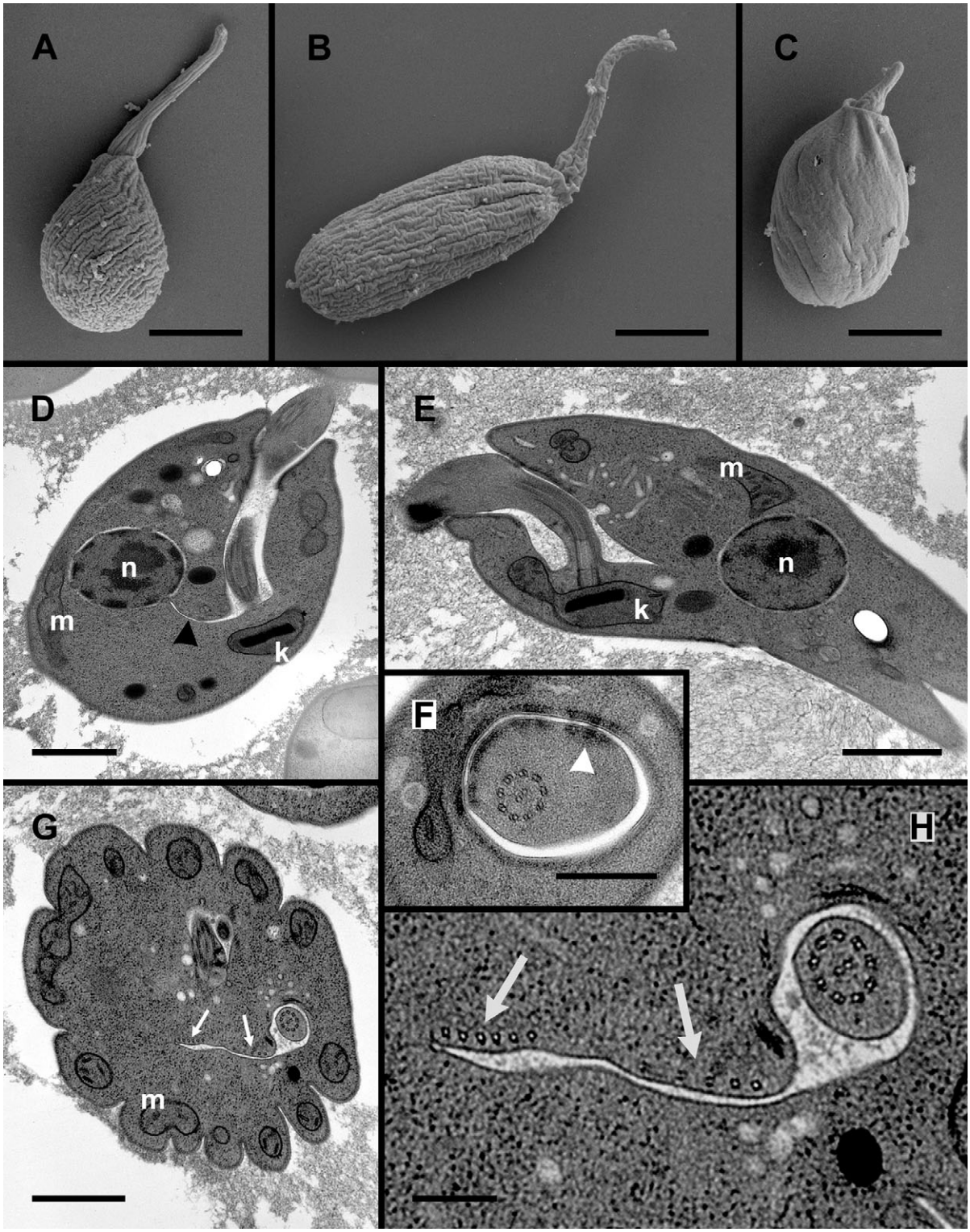

Figure 2 Scanning (**A**–**C**) and high-pressure freezing transmission (**D**–**H**) electron microscopy of *Herpetomonas tarakana* (D, E)—longitudinal sections of different morphotypes showing typical features of trypanosomatids (n = nucleus; k = kinetoplast; m = mitochondrion). Black arrowhead shows cleft-like invagination of flagellar pocket. (F) Cross-section of the flagellum inside flagellar pocket. Desmosomes in the contact area between flagellum and the membrane of the flagellar pocket are marked by white arrowhead. (G) Cross-section of the cell displaying cell ridges and mitochondrial branches. Microtubules of the reduced cytostome—cytopharyngeal complex lining cleft-like invagination of flagellar pocket are marked by white arrows. (H) A close-up of (G). Scale bars are 2 μm (A–E, G), 500 nm (F), and 250 nm (H).

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sp. G30 and G38, GenBank acc. nos JQ658820 and JQ658828, respectively) showed the same level of identity. Confirming our previous observation, the gGAPDH gene of OSR18 (GenBank acc. no. KR868692) was not as conserved and exhibited only 92% and 91% identity to its *Herpetomonas samuelpessoai* and *H. nabiculae* orthologs (Votýpka et al. 2014).

For phylogenetic reconstruction, we prepared a concatenated 18S rRNA + gGAPDH sequence alignment for species representing major trypanosomatid clades. The branching of single-gene trees was similar, with overall lower statistical support for the gGAPDH tree. The Bayesian and maximum likelihood trees based on a concatenated set were congruent and mainly consistent with previously published ones (Fig. 3). The isolate OSR18 grouped within a clade that also comprised *H. nabiculae*, *H. samueli*, and *H. samuelpessoai*. The second species of *Herpetomonas* represented by samples OSR27, OSR29 (in part), and OSR32 proved to be a sister to the *H. ztiplika* + *H. trimorpha* clade.

It needs to be noted that all *Herpetomonas* spp. with a notable exception of *H. mariadeanei* constitute a well-delineated monophyletic group with 100% bootstrap support. However, the latter species is quite distant from the rest of the genus demonstrating an intergeneric level of divergence. As for the relatively compact cluster of other herpetomonads, two closely related species *H. mirabilis* and *H. wanderleyi* occupied the basal position within it, though only with moderate statistical support. As in many previous reconstructions, the genera *Herpetomonas* and *Phytomonas* appeared as sister clades.

Experimental infection

To study host specificity, heterologous infection of cockroaches was attempted under the laboratory conditions. None of the wild cockroach species in which trypanosomatids were discovered (*E. lapponicus*, *Ph. chladeki*, *Ph. hungarica*, *Ph. maculata*) can be maintained in the laboratory for a reasonable period of time. Therefore, for in vitro studies three synanthropic species, *P. americana*, *N. cinerea*, and *B. germanica* were used. In all cases, the established infection was only transient, being successfully cleared by the host either by day 6 (*P. americana* and *B. germanica*) or day 12 (*N. cinerea*).

DISCUSSION

Herpetomonas Kent 1880 is the first described genus of monoxenous trypanosomatids. In traditional morphotype-based classification, the distinctive feature of this genus was the presence of opisthomastigotes, cells with the kinetoplast located posterior to the nucleus (Hoare and Wallace 1966). Due to their rarity in some species, opisthomastigotes can be easily overlooked. This resulted in a taxonomic confusion, with some true herpetomonads classified as *Leptomonas* or *Phytomonas* spp. and vice versa (Teixeira et al. 1997). At the same time the distinctness of the endosymbiont-bearing trypanosomatids, also with the posteriorly located kinetoplast, led to the creation of a separate morphotype—the opisthomorph, differing from opisthomastigotes in shape (Teixeira et al. 1997). The necessity of this distinction was dubious given the fact that the same opisthomorphs were described for the endosymbiont-free *H. samuelpessoai* as well (Roitman et al. 1976). Therefore, for a long time, the boundaries of this genus remained poorly defined and it was considered polyphyletic. Over a hundred species were formally described, but only few of them have withstood recent scrutiny (Borghesan et al. 2013; Podlipaev 1990). Dipterans are the most common hosts for *Herpetomonas* spp., although other groups, such as hemipterans, siphonapterans, and even ciliates were also implicated (Fiorini et al. 2001; Votýpka et al. 2013; Wallace 1966). Herpetomonads were also found in plants (Fiorini et al. 2001; Marin et al. 2007), though it is debatable whether plants serve as specific hosts or just represent an occasional temporary habitat for these flagellates. Even more remarkable is the fact that *Herpetomonas* sp. was isolated from an immunocompromised human, thus suggesting that at least some species have the capacity to adapt to high temperatures and harsh conditions associated with vertebrate blood (Morio et al. 2008). All the evidence stated above shows that herpetomonads represent a group of trypanosomatids that is extremely active in switching to new hosts. The ability of the isolate OSR18 (*H. tarakana*) to sustain elevated temperature shows its wide adaptive potential and makes it a good model for studying temperature adaptations in trypanosomatids.

Host specificity is one of the critical issues in any parasitological exploration. Applied to the particular case of the isolate OSR18, we suggest that wild cockroaches *E. lapponicus* and *Phyllodromica* spp. are specific hosts for this species. Our evidence is based on three facts: (i) while dipterans are the prevalent hosts of *Herpetomonas* spp. (Teixeira et al. 1997; Wallace 1966), *H. tarakana* has never been encountered in flies; (ii) this species clusters together with other "odd" *Herpetomonas* species: *H. nabiculae* (originally isolated from the bug *Nabis flavomarginatus*, Podlipaev 1985; Kostygov et al. 2011), *H. samueli*, and *H. samuelpessoai* (both from the predator reduviid bug *Zelus leucogrammus*, Carvalho and Deane 1974 or brachyceran dipterans, Týč et al. 2013); (iii) the host specificity of OSR18 is restricted to wild cockroaches from which it was isolated, as demonstrated by unsuccessful cross-infections (although, we cannot exclude the possibility that infection potential of the cultivated parasites can be substantially decreased during prolonged passaging in vitro). Moreover, the images of trypanosomatids encountered in wild cockroaches in the USA (belonging along with *Ectobius* and *Phyllodromica* to the family Blatellidae) capture cells that are similar to OSR18 (Semans 1939).

Thus, we describe herein a new species of the genus *Herpetomonas* that parasitize cockroaches. We propose to name it *H. tarakana* (see taxonomical section below). According to our results, trypanosomatids seem to be scarce in cockroaches; however, it is premature to gener-

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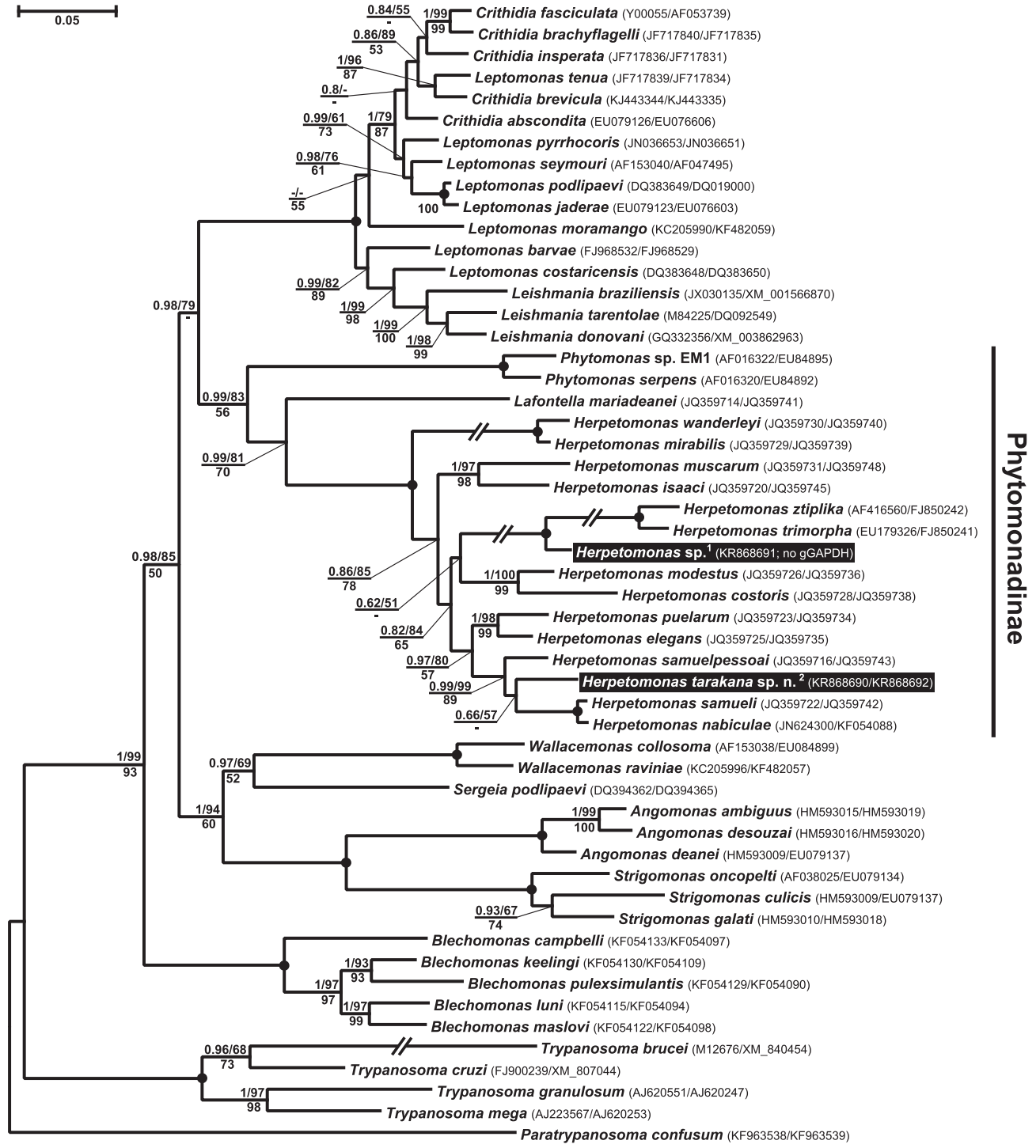

Figure 3 Phylogenetic tree of isolates studied in this work inferred by maximum likelihood method using 18S rRNA + gGAPDH concatenated set. The length of all branches is proportional to the number of substitutions per site. Double-crossed branches are at 50% of their original lengths. Numbers at nodes indicate posterior probability/bootstrap percentage (top), and expected likelihood weight (bottom). Values less than 0.5% and 50% are replaced with dashes. Nodes having 1.0 posterior probability, 100% bootstrap and 100% expected likelihood weight support are marked with black circles. The tree is rooted with *Paratrypanosoma confusum*. The bar represents number of substitutions per site. The species isolated from cockroaches are highlighted. [1]Represented by OSR27, OSR29 (in part), OSR32. [2]Represented by OSR18, OSR21, OSR26, OSR28, OSR29 (in part), OSR33, and OSR34.

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alize as less than a dozen out of about 4,600 host species were examined. Further assessment of the diversity of the trypanosomatid flagellates parasitizing cockroaches, especially in tropical regions, may lead to discovery of new interesting taxa within this group.

Meanwhile, some taxonomic changes are necessary for a better classification of already known species. The long phylogenetic distance between *H. mariadeanei* and the rest of the genus is at the level of intergeneric differences. Moreover, the discussed species has a unique morphological character—very long endomastigotes with even longer coiled flagellum within the cell, whereas other herpetomonads have only oval endomastigotes. Therefore, we propose to move this species from *Herpetomonas* to a newly erected genus, *Lafontella* (see taxonomical section below).

We believe that the exploratory nature of *Herpetomonas* spp. and especially the ability of some species to live in plants are in accord with the close relationship between the genera *Phytomonas* and *Herpetomonas* that has been observed in many previously published phylogenetic reconstructions (Borghesan et al. 2013; Votýpka et al. 2014). It is quite plausible that the ancestors of *Phytomonas* were *Herpetomonas*-like trypanosomatids that were "exploring" plants as their potential habitat. Our observation that *H. mirabilis and H. wanderleyi,* whose morphology (very long cells with the body twisted many times, Borghesan et al. 2013) is reminiscent of that of *Phytomonas* spp., are located near the base of the *Herpetomonas* clade, is also in agreement with this hypothesis. Importantly, in addition to phylogenetic affinity, these genera also share other common features. For example, they lack arginase (EC 3.5.3.1, arginine amidinase, canavanase, L-arginase, arginine transamidinase), an enzyme catalyzing the final step of the urea cycle (Wu and Morris 1998). This enzyme is present in Leishmaniinae, Strigomonadinae, and *Wallacemonas* but is absent from all investigated species of the genera *Herpetomonas* and *Phytomonas* (Camargo et al. 1978, 1987; Yoshida and Camargo 1978). Therefore, it is indispensable in our view to unite the three genera (namely *Herpetomonas,* the newly erected *Lafontella*, and *Phytomonas*) into the new subfamily Phytomonadinae (see taxonomical section).

TAXONOMIC SUMMARY

Class. Kinetoplastea (Honigberg, 1963) Vickerman, 1976
Subclass. Metakinetoplastina Vickerman, 2004
Order. Trypanosomatida Kent, 1880
Family. Trypanosomatidae (Doflein, 1901) Grobben, 1905
Subfamily. Phytomonadinae subfam. n. Yurchenko, Kostygov, Votýpka et Lukeš, 2015
Diagnosis. Clade of monoxenous parasites of insects (Diptera, Heteroptera, Dictyoptera, and Siphonaptera) and dixenous parasites of insects and plants defined by phylogenetic analyses based on 18S rRNA and gGAPDH gene sequences. Forms promastigotes, choanomastigotes, opisthomorphs, and long endomastigotes in culture, with promastigotes and choanomastigotes dominating. Arginase absent.
Type genus. *Herpetomonas* Kent, 1880
ZooBank registration. FFC1BA01-1336-4BEC-B7CE-1C56FD2B5E41
Etymology. The name of the subfamily has originated from the name of the most intensively studied genus of this clade, *Phytomonas* Donovan, 1909.

Genus *Phytomonas* Donovan, 1909

Type species. *Phytomonas davidi* (Lafont 1909) Donovan, 1909 [basionym: *Leptomonas davidi* Lafont 1909]

Genus *Lafontella* gen. n. Kostygov et Yurchenko, 2015

Type species. *Lafontella mariadeanei* (Yoshida, Freymuller et Wallace, 1978) Kostygov et Yurchenko, 2015 [basionym: *Herpetomonas mariadeanei* Yoshida, Freymuller et Wallace, 1978]
Genus composition. Lafontella mariadeanei comb. n. (Yoshida, Freymuller et Wallace, 1978) Kostygov and Yurchenko, 2015
Diagnosis. Is defined by unique position on the 18S rRNA-based phylogenetic tree. Does not cluster within either *Herpetomonas* or *Phytomonas*. Forms promastigortes, opisthomastigotes, and long endomastigotes with elongated coiled intracellular flagellum.
ZooBank registration. 23C781B6-ACDB-4D0D-A61D-1DDD8516CEA5
Etymology. The generic name honors Alexandre Lafont who discovered and described the first species of the genus *Phytomonas*, *Ph. davidi* (Lafont 1909).

Genus *Herpetomonas* Kent 1880

Type species. *Herpetomonas muscarum* (Leidy, 1856) Kent 1881 [basionym: *Bodo muscarum* Leidy, 1856]
Genus composition (validated species). *Herpetomonas muscarum* (Leidy, 1856); *H. costoris* (Wallace et al., 1965); *H. elegans* Teixeira et Camargo, 2012; *H. isaaci* Teixeira et Camargo, 2012; *H. mirabilis* (Roubaud, 1908); *H. modestus* Teixeira et Camargo, 2012; *H. nabiculae* (Podlipaev 1985); *H. puellarum* Teixeira et Camargo, 2012; *H. samueli* (Carvalho, 1973); *H. samuelpessoai* (Carvalho, 1973); *H. trimorpha* Zídková 2010; *H. wanderleyi* Teixeira et Camargo, 2012; *H. ztiplika* Podlipaev, 2004.

Herpetomonas tarakana Kostygov, Grybchuk-Ieremenko et Yurchenko sp. n. (Fig. 1, 2)

Species diagnosis and description. Forms promastigote, choanomastigotes, opisthomorphs and endomastigotes in culture, with promastigotes and choanomastigotes dominating. Longitudinal ridges are detectable on the cell surface. Cells in the culture range from 6.6 to 14 μm long and from 1.6 to 4.0 μm wide with flagella varying from 5.2 to 15.3 μm for elongate promastigotes and between 4.7 and 9.6 μm long and 3.6 to 6.2 μm wide with flagella siz-

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ing from 1.5 to 12.3 μm for spherical choanomastigotes. The kinetoplast disk is compactly packed and varies between 467 and 810 nm in length and 115 to 162 nm in diam. Can withstand elevated temperature.

Type host. *Ectobius lapponicus* (Dictyoptera: Blattodea).

Site. Intestine.

Type locality. Vicinity of Šilheřovice, Czech Republic (N49°54′16″ E18°16′28″).

Type material. The name-bearing type, a hapantotype, is a Giemsa-stained slide of the primary isolate OSR18, deposited in the research collection of the Life Science Research Centre, Ostrava, Czech Republic (accession code: 2015/OSR18/S). An axenic culture of the primary isolate OSR18 is deposited in the research collections of the Life Science Research Centre of the University of Ostrava, Department of Parasitology at Charles University in Prague, and in the Institute of Parasitology, České Budějovice, Czech Republic (accession code: OSR18).

ZooBank registration. C5FD6594-F242-4037-AAAB-EC6A5A7DE771

Etymology. The species name *tarakana* is derived from the word "таракан" \tarakan\ = cockroach in Russian, in accusative declension.

Gene sequences. KR868690 (18S rRNA) and KR868692 (gGAPDH).

Remarks. Based on the sequences of 18S rRNA gene, *P. chladeki* and *Ph. maculata* also serve as hosts for *H. tarakana*.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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