Worldwide Presence and Features of Flea-Borne *Rickettsia asembonensis*

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*Rickettsia asembonensis*, the most well-characterized rickettsia of the *Rickettsia felis*-like organisms (RFLO), is relatively unknown within the vector-borne diseases research community. The agent was initially identified in peri-domestic fleas from Asembo, Kenya in an area in which *R. felis* was associated with fever patients. Local fleas collected from domestic animals and within homes were predominately infected with *R. asembonensis* with <10% infected with *R. felis*. Since the identification of *R. asembonensis* in Kenya, it has been reported in other locations within Africa, Asia, the Middle East, Europe, North America, and South America. With the description of *R. asembonensis*-like genotypes across the globe, a need exists to isolate these *R. asembonensis* genotypes in cell culture, conduct microscopic, and biological analysis, as well as whole genome sequencing to ascertain whether they are the same species. Additionally, interest has been building on the potential of *R. asembonensis* in infecting vertebrate hosts including humans, non-human primates, dogs, and other animals. The current knowledge of the presence, prevalence, and distribution of *R. asembonensis* worldwide, as well as its arthropod hosts and potential as a pathogen are discussed in this manuscript.

**Keywords:** Rickettsia, *Rickettsia asembonensis*, flea-borne, worldwide distribution, arthropod hosts, *Rickettsia felis*-like organisms

**INTRODUCTION**

*Rickettsia asembonensis* is a Gram negative, obligate intracellular bacteria of the order Rickettsiales and family Rickettsiaceae (1). Among *Rickettsia* spp. with validly published names, it is most closely related to *R. felis* (Table 1) (4–6, 8, 9, 11, 13–15, 17–19, 30). However, among incompletely characterize rickettsiae, *R. asembonensis* genetically groups with other *R. felis*-like organisms (RFLO). The RFLOs are genetically related to *R. felis* but consist of a unique group of rickettsiae that are associated with various arthropods including fleas, ticks, mites, and tsetse flies for which limited knowledge of their biology and pathogenicity is available (3, 16, 31). Unfortunately, the genetic information of the majority of RFLOs in the GenBank database is fragmentary. Of the RFLOs described, only *R. asembonensis* (32) and “*Candidatus Rickettsia senegalensis*” (3) have been cultured (from *C. felis*) and characterized.

Other flea-borne rickettsiae include, besides the aforementioned *R. felis* and “Ca. *R. senegalensis,*” *Rickettsia typhi*, a member of the typhus group of rickettsiae (TGR). *R. typhi* is the causative agent of murine typhus, a febrile disease that is found throughout the world. *R. typhi* is vectored by various flea species-especially *X. cheopis*, but also other *Xenopsylla*...
TABLE 1 | Worldwide distribution of *Rickettsia asembonensis* and closely related, incompletely characterized rickettsiae.

| Rickettsial agents | Source | Country | Location | Sequence comparison with *R. asembonensis* NMRCii (%) | Year in | References |
|--------------------|--------|---------|----------|---------------------------------|--------|-----------|
| Rickettsia felis   | Ctenocephalides felis | USA | California, El Labs at Soquel | rrs 99.5, gltA 98, ompA 92.5, ompB 94.7, sca4 95.7, 17kDa 97.3 | 1999 | (2) |
| Rickettsia sp. RF2125 | Ctenocephalides canis | Thailand | Sangkhlaburi District, Kanchanaburi province | – 99.3, – 99.7, – – | 2002 | (4) |
| Rickettsia sp. cf1and5 | Ctenocephalides felis | USA | Greenville County, South Carolina | – 99.5, – – – – | 2005 | (5) |
| Rickettsia sp. SE313 | Echidnophaga gallinacea | Egypt | Mansoura, Zagazig | – 99.7, – – – | 2005 | (6) |
| Rickettsia sp. c9 | Ctenocephalides felis | USA | Not provided | – – 99.9, 99.8 – | 2006 | Reeves et al., (Unpubl.) |
| Rickettsia sp. FS27 | Orchopeas horwadi | USA | Not provided | – 99.7, – – | 2006 | Reeves et al., (Unpubl.) |
| Rickettsia sp. | Ornithonyssus bacoti | Egypt | Ebsheawy, El Quseir, Qara Oasis, Zagazig, Arab El Maamal | – – 99.7 – | 2008 | (8) |
| Uncultured R. sp. Clone H56-2 | Archaeopsylla enriettai | Germany | Bavaria | – – 100 – | 2009 | (9) |
| Uncultured R. sp. Clone ARV5606 | Ctenocephalides felis | Peru | Iquitos | – 99.7 – – – | 2011 | (11) |
| Rickettsia sp. RF2125 | Pulex irritans | Hungary | Various parts of the country, specific information not provided | – >99.3 – – | # (7) |
| Rickettsia sp. R14 | Ceratophylus fasciatus | India | Not provided | – 99.7 – 99.9 – | 2010 | Chahota et al., (Unpubl.) |
| R. endosymbiont of C. felis isolate F143 | Ctenocephalides felis | Thailand | No specific information provided (45 Districts) | – 99.7 – – – – | 2011 | (11) |
| R. endosymbiont of C. felis isolate F144 | Ctenocephalides felis | Thailand | No specific information provided (45 Districts) | – 99.7 – – – – | 2011 | (11) |
| Rickettsia sp. clone 4-G/G/JP-10-2 | Ctenocephalides felis | Costa Rica | Limon (Guacimo) | – 99 – – – – | 2011 | (12) |
| Rickettsia sp. | Synosternus pallidus | Senegal | Dielmo | – 100 – 100 – | 2011 | (13) |
| Rickettsia asembonensis F30 | Ctenocephalides canis | Kenya | Nyanza | 100 100 99.9 99.9 100 100 100 | 2011 | (14) |
| Rickettsia asembonensis F82 | Ctenocephalides felis | Kenya | Nyanza | 100 – 99.9 – 100 100 100 | 2011 | (14) |
| Uncultured R. sp. Clone HL2a | Ctenocephalides felis | Malaysia | Kuala Lumpur, Selangor | – 99.7 – – – | 2013 | (15) |
| Rickettsia sp. RFLO-18 | Ctenocephalides felis | Thailand | Was not deposited in the GenBank | – – 99.7 – – | # (16) |

(Continued)
| Rickettsial agents                  | Source                  | Country | Location          | Sequence comparison with *R. asembonensis* NMRCii (%) | Year in | References               |
|------------------------------------|-------------------------|---------|-------------------|------------------------------------------------------|---------|--------------------------|
|                                    | Rickettsia sp. J28p     | Peru    | Not provided      |                                                      | 2015    | Palacios-Salvatiera et al. (Unpubl.) |
|                                    | Rickettsia strain from C. felis | Ecuador | Pastaza           | 99.9 99.7 – 100 100 100 # (17)                        | 2014    | (18)                     |
|                                    | Rickettsia sp. Clone X | Xenopsylla ramesis | Israel | Negav | 100 99.7 100 100 – 100 100 # (17) | 2014 | (19) |
|                                    | Rickettsia sp. 9AL      | Ctenocephalides felis | Colombia | Villeta | 100 100 – 100 – – 100 # (17) | 2014 | (20) |
|                                    | Rickettsia sp. 0095     | Macaca fascicularis | Malaysia | Not provided | – 100 – 99.9 – – 100 100 100 # (17) | 2014 | (17) |
|                                    | Rickettsia sp. Clone Mai | Homo sapiens | University Malaya Medical Center | – 99 – 99.9 – – 100 100 100 # (17) | 2015 | (21) |
|                                    | Uncultured Rickettsia sp. Isolate F1 | Ctenocephalides felis | South Africa | Mpumalanga Province | 100 – – – – – 100 100 100 # (17) | 2015 | (22) |
|                                    | Rickettsia asembonensis 0-TP-1 | Ctenocephalides felis | Costa Rica | Cahuita, La Virgen, Limon, Tulaiba, Guapiles | – 99.7 – – – – 100 100 100 # (17) | 2016 | (23) |
|                                    | Rickettsia asembonensis 6-CP-4-1 | Pulax simulans | Costa Rica | Cahuita, La Virgen, Limon, Tulaiba, Guapiles | – 99.7 – – – – 100 100 100 # (17) | 2016 | (23) |
|                                    | Rickettsia asembonensis 6-CP-4-4 | Ambylyomma ovale | Costa Rica | Cahuita, La Virgen, Limon, Tulaiba, Guapiles | – 99.7 – – – – 100 100 100 # (17) | 2016 | (23) |
|                                    | Rickettsia asembonensis CF268US | Ctenocephalides felis | USA | Orange County, California | 99.9 99.7 99.9 99.9 100 – 100 100 100 # (17) | 2016 | (24) |
|                                    | Rickettsia asembonensis Tapes | Rhipicephalus sanguineus | Brazil | Tapes | – 99.6 – – – – 100 100 100 # (17) | 2016 | (25) |
|                                    | Rickettsia asembonensis SP003-M | Ctenocephalides orientis | Malaysia | Kuala Lumpur, Perak, Johore, Kelantan, Pahang, Negeri Sembilan | – 99.2 – – – – 100 100 100 # (17) | 2016 | (26) |
|                                    | Rickettsia asembonensis DB32B | Rhipicephalus sanguineus | Malaysia | Kuala Lumpur, Selangor, Pahang | – 99.6 – – – – 100 100 100 # (17) | 2017 | (27) |
|                                    | Rickettsia asembonensis GF#68 | Ctenocephalides felis | Brazil | Maramhao State | – 99.6 – 99.9 – 100 100 100 # (17) | 2017 | (28) |
|                                    | Rickettsia asembonensis F30 | Ctenocephalides felis | Uganda | Southwestern Uganda | – 100 – 99.6– 100 100 100 # (17) | 2017 | (29) |
|                                    | Rickettsia asembonensis 7.2 | Ctenocephalides felis | USA | Galveston, Texas | – – – – – – 100 100 100 # (17) | 2018 | (29) |
|                                    | Rickettsia asembonensis VGD7 | Ctenocephalides felis | Peru | Peruvian Amazon | – 99.8 99.8 100 99.8 100 100 100 # (17) | 2017 | (30) |

*Rickettsia felis* and *Candidatus Rickettsia senegalensis* are provided as reference rickettsiae that are closely related to but distinct from *Rickettsia asembonensis*.
species such as X. astia and X. bryaiiensis (33, 34), Synosternus pallidius, and rarely, but importantly, Ctenocephalides felis the common cat flea that readily parasitizes cats, opossums, and other domestic, peri-domestic, and wild animals. C. felis is believed to be capable of hosting R. typhi and to vector murine typhus in areas outside the traditional range of rat fleas and rats (35, 36).

R. felis, R. asembonensis, and “Ca. R. senegalensis” fall within the spotted fever group rickettsiae (SFGR) that genetically clusters within the transitional group of rickettsiae (37). R. felis is associated with flea-borne spotted fever (38, 39) and the pathogenicity of R. asembonensis and “Ca. R. senegalensis” is currently unknown. These three agents have worldwide distribution, are often sympatric and most often found parasitizing cat and dog fleas (3, 4, 14, 38, 40, 41).

“Candidatus R. senegalensis” was first described in C. felis fleas from Senegal (3) and an agent believed to be “Ca. R. senegalensis”-like (Rickettsia sp. RF31) had been detected previously in C. felis near the Thailand-Myanmar border (4). A very close genetic relationship (99.9% based on gltA gene sequence) between Rickettsia sp. RF31 and the latter is notable (3). “Ca. R. senegalensis” is distinct from, but can be sympatric with, R. felis and R. asembonensis (40). It has worldwide distribution but is not reported as often as R. felis or R. asembonensis. Reports of its molecular presence in cat tissues suggests it may be able to infect vertebrate animals (41).

**HISTORY OF RICKETTSIA ASEMBONENSIS**

Incompletely characterized rickettsiae with various identities most closely related to R. asembonensis populated the literature in the early 2000s (Table 1). These agents were detected by molecular techniques [i.e., PCR, nested PCR (nPCR), and/or quantitative real-time PCR (qPCR)] and then characterized by sequencing different size fragments of one or more commonly used gene targets (rrs, gltA, ompA, ompB, sca4, or the 17 kDa antigen gene). The first agent, referred to as Rickettsia sp. RF2125, was detected in Ctenocephalides canis in western Thailand near the Myanmar border (4). The agent was characterized by the sequence of a 1,171 bp fragment of the gltA that showed the rickettsial agent to be unique but most closely related to R. felis (4). The sequence of a 790 bp fragment of ompB (JX183538) from the original Rickettsia sp. RF2125 DNA preparation was obtained at that same time as the gltA but was not reported in the original article (4). It was reported in 2013 (14). We believe that RF2125 may have been the first detection of R. asembonensis or a very similar agent. Additional reports of R. asembonensis or an agent closely related to it continued to occur worldwide (Figure 1) shortly thereafter including: Rickettsia sp. cf1 and 5, USA (5); Rickettsia sp. SE313, Egypt (6); Rickettsia sp. H56-2, Germany (8); Rickettsia sp. ARV5606, Peru (9); and Rickettsia sp. Synosternus, Senegal (13). These partially characterized agents were described prior to our complete characterization of R. asembonensis (1). These agents are summarized along with R. asembonensis to include their distribution, vector hosts, and genetic characterization (see Table 1).

R. asembonensis was initially described as an unknown Rickettsia sp. detected in various flea species (i.e., C. felis, C. canis, Echidnophaga gallinaceae, X. cheopis, and Pulex irritans) collected from various domestic animals (i.e., dogs, cats, and rodents) and houses (by light traps) in Asembo, Kisumu, in western Kenya during an epidemiologic surveillance study (14). This study was conducted concurrently with a fever study in which the presence of R. felis was identified in 7.2% of febrile patients (42). The initial molecular characterization of the R. asembonensis agent was accomplished utilizing a multilocus sequence typing (MLST) algorithm (43). Prevalence of this new agent (~91.7%) in collected fleas was found to be distinctly different from that of R. felis (8.3%) (14).

Subsequently, additional fleas collected from the same hosts and locations within the livestock-owning compounds in Asembo were processed for rickettsial culture. The new agent, Rickettsia asembonensis NMRCii, was successfully cultured from a pool of five individual flea triturate cultures isolated from C. canis and C. felis fleas obtained from domestic dogs. The cultures were initially grown in S2 and subsequently in C6/36 cell lines at 25°C (32), but not in Vero and L929 cell lines or embryonated chicken eggs incubated at 37°C (1).

The culture of R. asembonensis NMRCii was analyzed by microscopy, including Diff-Quik/acridine orange staining and transmission electron microscopy (32). The R. asembonensis were observed in the Drosophila S2 and Aedes albopictus C6/36 cell lines as early as 3 days post-infection, and could be observed at multiple time points throughout the average culture time of 40–45 days (32). Rickettsiae were observed both intra- and extracellularly at time points ranging from 15 to 30 days throughout the course of the continuous culture (32). The new agent was observed by acridine orange staining in singlets, doublets, and during heavy parasitization of host cells, in long chains (32). Transmission electron microscopy of the R. asembonensis revealed multiple free rickettsiae (round to elongated morphology) in the cytoplasm of the host cells, with normal rickettsial size [diameter 0.375–0.5 µm (round morphology), length 0.5–0.625 µm, width/diameter 0.25–0.375 µm (elongated morphology)]. A cell wall membrane, defined periplasmic space, and cytoplasmic membrane were observed, as well as the electron lucent “halo” (rickettsial slime layer) (32). Intranuclear localization/growth of the agent was not detected by acridine orange or by transmission electron microscopy (32).

Genetic characterization of the cultured R. asembonensis NMRCii by MLST using rickettsial genes rrs, gltA, ompA, ompB, and sca4; plasmid analysis; and whole genome sequencing confirmed that the new agent was indeed a unique Rickettsia species (1, 44). R. asembonensis NMRCii was shown to have an estimated genome size of 1.40 Mb, possessed a 21,692 bp circular plasmid and had a G+C content of 32.2%. The R. asembonensis plasmid, pRAS01, was discovered to be unique as it only shared 89% homology with that of R. africae ESF5 and only 84% homology with that of R. felis. The R. asembonensis genome has 1,147 predicted protein-coding genes, 33 tRNA genes, and three
rrn operons. These characteristics are similar with those found within the genome of R. felis (NC_007109), which is 1.49 Mb in size and contains 1,400 protein-coding genes, 33 tRNA genes, and three rrn operons. Of the R. felis proteins, 1,157 (83%) have homologs in R. asembonensis (1, 44).

The sequences of R. asembonensis NMRCii, were 100% identical to those previously described for “Ca. R. asembonensis” isolates F30 and F82 for the following genes: rrs, gltA, sca4, and the 17kD antigen gene. For the ompA and ompB genes, the R. asembonensis NMRCii shared 99.86 and 99.98% similarity respectively, with the “Ca. R. asembonensis” isolates F30 and F82. The differences observed were as a result of nucleotide substitutions in two positions for the ompA gene and in one position for the ompB gene. A molecular phylogenetic analysis using 4,130 bp sequence of the variable gene-ompB open reading frame was conducted and the phylogenetic relationship between Rickettsia asembonensis NMRCii with R. felis, Rickettsia sp. PU01-02 (“Ca. R. senegalensis”) and other recognized Rickettsia species was determined (Figure 2).

Rickettsia asembonensis NMRCii was deposited in two separate culture collections (=DSM 100172 and =CDC CRIRC RAS0013) and the name officially changed (according to the rules of the International Journal of Systematics and Evolutionary Biology) from “Candidatus Rickettsia asembonensis” to Rickettsia asembonensis (1).

**ARTHROPODS ASSOCIATED WITH RICKETTSIA ASEMBONENSIS**

R. asembonensis DNA has been detected in various arthropods, but most commonly in fleas (Table 1). It has been identified in fleas from three families namely the Pulicidae, Ceratophyllidae and Coptopsyllidae. In the cosmopolitan Pulicidae family it has been associated with seven genera: Ctenocephalides (C. felis, C. canis, and C. orientis); Xenopsylla (X. cheopis, X. ramesis, and X. gerbilli); Archaeopsylla (A. erinacei); Echidnophaga (E. gallinacea); Pulex (P. irritans); and Synosternus (S. pallidus). In the family Ceratophyllidae, R. asembonensis has been detected in three genera: Ceratopsylla (C. fuscata); Orchopeas (O. howardi); and Nosopsyllus (N. laeviceps) and in one genus in the family Coptopsyllidae: Coptopsylla (C. lamellifer) (45).

High prevalence rates of R. asembonensis have been reported in C. felis and C. canis (sympatric species), S. pallidus, X. ramesis, and X. gerbilli with up to 95, 95, 91.4, 100, and 33.3% of the fleas positive for R. asembonensis, respectively (13, 14, 18, 40, 46). Similar results in Costa Rica and Brazil confirm the high prevalence of R. asembonensis in C. felis (23, 28). In addition, R. asembonensis has been associated with other fleas, usually in much lower prevalence than in the aforementioned fleas. These include E. gallinacea, P. irritans, C. lamellifer, X. hirtipes, and N. laeviceps. Often these fleas are positive for R. asembonensis in the same areas as fleas highly infected with R. asembonensis (14, 46). The presence of the R. asembonensis in minimally infected flea species may be due to co-feeding and not that these fleas are reservoir hosts for R. asembonensis. Other arthropods in which evidence of R. asembonensis has been found include the tropical rat mites (Ornithonyssus bacoti) in Egypt (7) and ticks (Amblyomma ovalae and Rhipicephalus sanguineus) (23, 25–27).

**PATHOGENICITY**

In limited laboratory studies no marked cytopathic effects were observed in S2 and C6/36 cells, beyond lysis of overly parasitized host cells (32). Additionally, no growth was observed in embryonated chicken eggs (1). Moreover, in two febrile studies conducted in Kenya no molecular evidence of this agent in
patients’ blood was seen whereas *R. felis* DNA was detected in 3.7 and 7.2% of fever patients’ blood (42, 47). However, there is molecular evidence of *R. asembonensis* in a patient from Malaysia with fever, myalgia, arthralgia, mild headache, conjunctival suffusion, and the presence of petechiae noted on his limbs. Molecular analysis (gltA and ompB sequences) of the patient’s blood identified *R. sp. RF2125* (21). In addition, in the blood from a healthy free range domestic dog from Mnisi community situated in the northeastern corner of the Bushbuckridge Municipal Area, Mpumalanga Province, South Africa *R. asembonensis* was detected by NGS (22). Lastly, 12 of 50 healthy monkeys from Peninsular Malaysia had molecular evidence (100% gltA sequence similarity) of *R. sp. RF2125”* (20). Thus, from the mixed results presented, the question of pathogenicity for humans and other animals is not yet resolved and requires more investigation.

**FUTURE RESEARCH DIRECTION**

*R. asembonensis*-genotypes have been described in various biting and non-biting arthropods. Apart from *R. asembonensis* NMRCii that has been isolated in cell culture and whose full genome sequence is available in the GenBank Database, many of the others are just molecular isolates derived from arthropods with very limited sequence data for comparison. Functional and structural analysis of *R. asembonensis* is needed to ascertain differences and/or similarities between it and other rickettsial species. Moreover, research concerning the known/potential hosts of *R. asembonensis*, its current/potential arthropod vectors (both common and non-common), and its potential for interference with other rickettsial flea-borne pathogens (*R. felis* and *R. typhi*), as well as non-rickettsial pathogens such as *Yersinia pestis*, will be crucial to fully defining its pathogenicity and probability as a public health concern/nuisance across the world.

**AUTHOR CONTRIBUTIONS**

All authors contributed to the conception and design of the review. AM wrote the first draft of the manuscript. JJ, AL-F, HS, CF, and AR wrote revisions of the manuscript. All authors contributed to the manuscript’s final version, and read and approved the submitted version.

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