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We are connected: flea–host association networks in the plague outbreak focus in the Rift Valley, northern Tanzania

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Abstract

Context. Plague is a serious health problem in northern Tanzania, with outbreaks since 2008 in two districts located in Rift Valley. There is dearth of knowledge on diversity of small mammal and flea fauna occurring in this plague focus. Knowledge on interactions between fleas and rodent species that harbour the plague bacterium, *Yersinia pestis*, is important for developing strategies for control and prevention of plague.

Aims. This study aims to show how rodents and fleas are associated with each other in the plague focus.

Methods. Animals were trapped bimonthly from 2009 to 2012 in different habitats. The fur of animals was brushed to collect fleas, which were identified and quantified. Network analysis methods, randomisation and rarefaction curves were used to show how hosts and fleas are associated.

Key results. Thirteen species of rodents were associated with 26 species of fleas of which *Dinopsyllus lypusus, Xenopsylla brasiliensis* and *X. cheopis* are confirmed efficient vectors of *Y. pestis.* Randomisation and rarefaction curves established that *Lophuromys flavopunctatus* had significantly higher flea species richness ($n = 9$) than did all other hosts, whereas *Xenopsylla cheopis* and *Dinopsyllus* spp. showed greater host species richness than did other species of fleas. There was no significant correlation between host sex and flea abundance ($\chi^2 = 0.8$, d.f. = 6, $P = 0.371$), but significant differences between reproductive states (adults had more fleas than did subadults) were observed, which probably reflected typical positive correlation between size and flea abundance ($\chi^2 = 4.1955$, d.f. = 1, $P = 0.040$).

Conclusions. The plague outbreak focus in northern Tanzania has a diverse fauna of rodents and fleas with multiple patterns of association and connectivity.

Implications. Existence of diverse populations of rodents associated with a large number of flea species, some of which are efficient plague vectors, increases the potential for persistence and transmission of plague to humans in northern Tanzania.

Additional keywords: Karatu, Mbulu, rodents, *Yersinia pestis*.

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Introduction

Plague is a zoonotic disease, essentially of rodents, caused by the bacterium *Yersinia pestis*. It is primarily transmitted from infected rodents to humans by fleas. The flea ectoparasites of mammals usually form a strong association with the individual host. Flea species infesting rodents sometimes show specificity for certain genera or families (Krasnov and Khokhlova 2001). Fleas are able to locate a host using stimuli such as host body warmth, air movements, substrate vibration and odour of potential hosts or their products (Durden and Hinckle 2009). Flea species composition in a particular habitat is determined not only by host species composition but also by some properties of the habitat itself (Krasnov *et al*. 1997). Fleas are usually associated with the body of the host and the nests and, therefore, they can be transferred from one host to another through occupation of the same habitat or visitation of burrows of the same or different species (Marshall 1981). It has been reported that social contacts also enable flea exchanges within and between species (Krasnov and Khokhlova 2001). Patterns of flea infestation of their rodent hosts in northern Tanzania have been shown to vary due to seasonal and spatial differences in local microclimate (Laudisoit *et al*. 2009*a*). However, it has been argued that ectoparasite species composition is determined by both host species composition and habitat properties, but variations in flea species composition between localities in the tropics seem to be explained better by host species composition (Laudisoit *et al*. 2009*b*).

In areas with active plague infections, transmission of *Y. pestis* among rodents is facilitated by flea transfer between hosts, resulting in an enzootic plague cycle of the disease (Bevins *et al*. 2012). In the epizootic cycle of the disease, transmission to humans occurs primarily by the bites of fleas carrying the pathogen from rodents. This cycle becomes more complex when fleas transfer *Y. pestis* from wild rodents (sylvatic plague) to peri-domestic and domestic rodents, including commensal rodents that carry flea species that can transmit the disease agents to humans (Laudisoit *et al*. 2010; Leirs *et al*. 2010). Increased population of fleas and greater abundance of their rodent hosts will increase the chances of the transfer of *Y. pestis* from rodents to humans as a result of more frequent interactions. Indeed, most studies agree that ecological systems with differences in topography, soils, vegetation types and climate influence the diversity of host species, resulting in differences in the species composition and abundance of flea communities (Durden and Hinckle 2009), which could thus determine the transmission and persistence of plague in an area (Gage and Kosoy 2005; Laudisoit *et al*. 2009*a*; Laudisoit *et al*. 2009*b*).

Plague is enzootic in wild rodent populations in several localities in Tanzania (Kilonzo and Mtoi 1983; Kilonzo *et al*. 2005). In recent studies, evidence of *Y. pestis* DNA in rodents in the plague active foci in Mbulu and Karatu districts in northern Tanzania was reported (Ziwa *et al*. 2013). Previous studies in the area have shown that several species of rodents were serologically positive for *Y. pestis* F1 antigen during plague outbreaks (Makundi *et al*. 2008). Unfortunately, studies regarding the diversity of fleas and their interaction with their rodent hosts, which may shed light on how plague spreads from wild rodents to humans, are largely inadequate. Previous studies in plague-endemic areas in Tanzania primarily provided taxonomic lists of hosts and their ectoparasite species (Msangi 1969; Kilonzo 1976; Kilonzo and Mhina 1983; Makundi and Kilonzo 1994); however, little has been published on how these are connected. Studies on the abundance of fleas and their distribution among rodent hosts have indicated an overall flea index of 2.4 fleas per rodent host in Mbulu District (Haule *et al*. 2013), but the study was based on a few hosts and flea species and the authors did not show how the ectoparasites were associated with different host species.

In some of the active plague foci in Tanzania, changes to the habitats caused by human activity have led to the displacement of rodents, creation of new ecotones and, hence, increasing densities and interactions with them and their fleas (Makundi *et al*. 2003, 2005, 2009). Recent studies have suggested that land use could affect the risk of local transmission of plague (McCauley *et al*. 2015).

To understand the potential pathways of *Y.pestis* transmission from rodents to humans via fleas, a better understanding of the connections between them is necessary. Network analysis provides a useful tool for determining the patterns of association between parasites and hosts (Poulin 2010), but has never been used in the past to establish flea–host–habitat association patterns in plague active foci in Tanzania. The present study aims at establishing the association patterns of rodents and fleas in their natural habitats and potential effect in rodent plague dynamics.

Materials and methods

Study area

The study was conducted in two districts located in Rift Valley, northern Tanzania, namely Mbulu (04°4.5′S, 35°36′E) and Karatu (03.10°4.00′S, 34°47′E). The study areas are inhabited by the same ethnic group, the Iraqw, a Cushitic-speaking people of Afro-Asiatic origin.

The study was conducted between February 2009 and April 2012. In Karatu, rodent trapping was conducted in fallow land, cultivated fields and forest. Specific localities were Kilima Tembo, Kambi ya Simba Forest (03°16′13″S, 35°48′94″E); 1580 m a.s.l.), Kambi ya Nyoka (fallow land), Rhotia Kati (peri-domestic and in houses) and Amazing Tanzania Farm (cultivated area). In Mbulu, trapping was undertaken in several localities in the Division of Dongobesh (04°04′S, 35°22′E) at altitudes ranging from 1930 to 2250 m a.s.l. The following localities were sampled: Nahsay (fallow and cultivated land), Mongahay (natural moist forest, cultivated land, houses and fallow land), Arri (Nowu moist forest), Dawudi (Marang moist forest) and Mangisa (bush) (03°59′52.90″S, 35°21′34.65″E; 1995 m a.s.l.).

Description of habitats

The moist forest habitat consisted of closed canopy of tall and medium-height trees of different species, with a dense undergrowth of herbaceous plants, liana of different species and shrubs.

Cultivated land consisted of farmland planted with maize (*Zea mays*) intercropped with common beans (*Phaseolus vulgaris*). The unweeded-farm notable vegetation was mainly herbs of different species. Common herbs in farmland included *Amaranthus spinosum*, *Solanum incunum*, *Calylsea abyssinica*, *Coelina benghalensis* and *Sesa angolense.*

The fallow land from previous-year cultivation was mainly dominated by grasses. Major grass species included *Cynodon dactylon*, which was the most dominant. Other grass species included *Brachiaria eruciforis*, *Digitaria milanjianus* and *Tragus berteronianus*. Vegetation cover in fallow land also changed depending on the season (wet or dry) and the intensity of grazing by cattle, goats and sheep.

The bush habitat had few scattered trees, shrubs and bushes. The most conspicuous grass species were *Hyperrhenia ruffa*, *Sorghum versicolor*, *Cynodon dactylon*, *Digitaria milanjianus* and *Eragostis trichophora.* The main woody species in the bushes included *Albizia amara*, *Acacia albida*, *Leucaena leucocephala*, *Acacia tortilis*, *A. xanthophloea* and *Sesbania bispinosa*.

Peri-domestic areas were surroundings located 5–10 m from houses and were either cropped with maize and beans or were fallow during the study period.

The domestic environment consisted of the house and the immediate surroundings (0–5 m from the house), which were usually not cultivated.

Animal trapping and handling

Animals were live-trapped bimonthly from February 2009 to April 2012. We used Sherman traps baited with peanut butter mixed with maize flour. In each of the study habitats, 100 traps

were set in 10 trap lines, each consisting of 10 trapping stations (10×10) 10 m apart for three consecutive nights and were checked every morning. In peri-domestic areas, the number of traps and how they were set depended on the configuration of the surroundings. Typically, we set 10–20 traps in the surroundings of a homestead and five traps in houses. Captured animals were taken to a field laboratory, and processed as described in elsewhere (Laudisoit *et al*. 2009*a*). Identification of animals followed Kingdon's field guide for African mammals (Kingdon 1974).

Ethical considerations

Researchers observed and complied with laws, regulations and policies on humane care and handling of animals for research purposes guided by the Code of Conduct for Research Ethics of Sokoine University of Agriculture, Tanzania, and Tanzania's *Animal Welfare Act* of 2008.

Dataset composition

Data from all trapping sessions were used for analyses. Because of logistical limitations, it was not possible to identify all fleas found on all rodents, and the dataset included rodents of which the fleas were identified, together with rodents with unidentified fleas and rodents without fleas. It was necessary to include rodents for which no fleas were found for correct analyses of flea abundance and richness. So as to correctly exclude rodents with unidentified fleas while including rodents without fleas, we used the following method. First, we calculated the proportion of flea-positive rodents for which the fleas were identified. The same proportion of rodents on which no fleas were found was randomly chosen to be included in the final dataset.

Network analysis

We used network-analysis methods (Poulin 2010) to show in one graph how flea species and their hosts are associated. Every host and flea species was a point (node) in the graph, and for each flea that was observed on a host species, a connection (edge) was drawn between the two. Rodents without fleas were connected with 'no fleas' node. For each node, we indicated the proportion of habitats in which the corresponding species was found. For network analysis, habitats were grouped into the following three: domestic (sampled in domestic and peri-domestic habitats), field (cultivated land and fallow land) and forest (bush and forest).

We used a linear regression analysis to test whether the number of host individuals (log-transformed) caught correlated with the number of flea species found on the host species. A generalised linear mixed-effects model with log-link function (for Poisson distributed data) was used to test for correlations between richness or abundance and among habitat, reproductive age and sex, where these three variables were treated as random effects, and species was included as random effect to account for inter-species differences between sample size and habitat.

We used a standard method involving randomisation and rarefaction, a statistical method to estimate and compare richness (Krebs 2009), that takes into account the sample size and calculates the rarefaction (or species accumulation) curves. The rarefaction curves estimated the expected number of species for a range of sample sizes, which was obtained by bootstrapping. For instance, if 90 *Mastomys natalensis* individuals had about nine flea species, then it would be possible to intrapolate how many flea species would be detected if only 10 *M. natalensis* individuals were sampled. To estimate this, 10 individuals were randomly chosen on which the number of flea species was counted. This step was repeated (1000 iterations), and the average number of species was taken, with 95% confidence interval. This was performed for the whole range of sample sizes between 1 and 116 individuals, which resulted into the rarefaction curve.

Two-by-two comparisons of flea species richness and host species richness

We used the rarefaction data to compare the flea species richness of any two rodent host species. For each combination of host species, the minimum possible number of individuals was chosen, which was the maximum for the species with the least number of captured individuals (e.g. if Host species A had 10 individuals, and Host species B had 20, the rarefaction data were used for 10 individuals for both species). By using a randomisation test, flea species richness was repeatedly compared (1000 iterations), and the proportion of times that Individual A had more flea species than did Individual B, was recorded. This produced the *P*-value. Host species richness (the number of flea species found on a host) was tested in the same way.

All data manipulation, bootstrapping, randomisation, statistics and plotting were undertaken using R (R core Team 2013) and R packages network 1.9.0 (Carter *et al*. 2014*a*), sna 2.3.2 (Carter *et al*. 2014*b*) and plotrix (Lemon 2006).

Results

In total, 943 small mammals were captured in different habitats in the two districts comprising of 13 species of rodents (4 individuals of an unidentified species cfr *Aethomys*), several species of *Crocidura* and one elephant shrew (*Petrodromus* sp.; Fig. 1).

Mastomys natalensis dominated the captures (37.5%) in fallow and cultivated land, whereas *Lophuromys flavopunctatus* and *Praomys delectorum* were most dominant in the moist forests (15.6% and 14.1%) respectively. These three species represented more than 66% of all rodents and shrews captured. Very few individuals of *Rattus rattus* (1.5%) were captured, which was attributed to rodent-control campaigns and various hygienic measures in houses during the 2008–2011 plague epidemics in the two districts.

A diverse assemblage of fleas consisting of 26 species (Fig. 2) was collected from 576 rodents and shrews. About 61.1% of the small mammals were infested with a total load of 749 fleas. Seven species of fleas were the most prevalent, namely *Ctenophthalmus calceatus* spp., *Ctenophthalmus* sp., *Dinopsyllus grypurus*, *D. lypusus*, *Pulex irritans*, *Xenopsylla brasiliensis* and *X. cheopis*. *Dinopsyllus grypurus*, *D. lypusus* and *X. brasiliensis* represented more than 50% of all collected fleas (Fig. 2).

The network graph shows clearly which hosts are connected with which fleas, and also which fleas are shared by host species in different and similar habitats (Fig. 3). Each host was given a different connection colour, so that it is easier to see how they

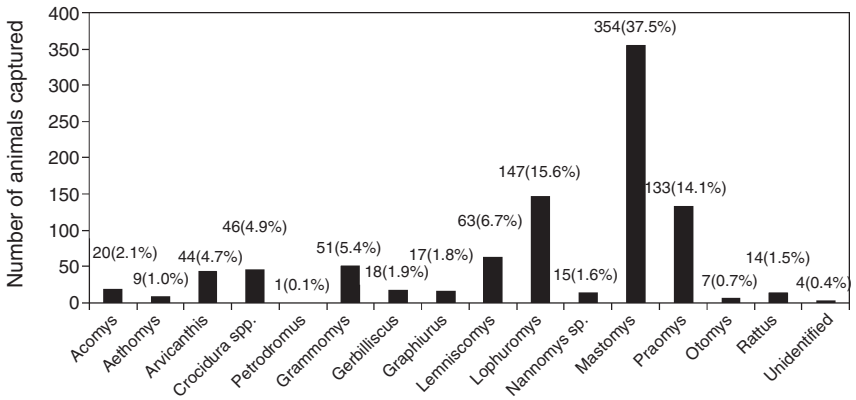

Fig. 1. Numbers and percentage of total of each species of rodents, *Crocidura* and elephant shrew (*Petrodromus* sp.) captured in the Rift Valley Districts of Mbulu and Karatu, northern Tanzania.

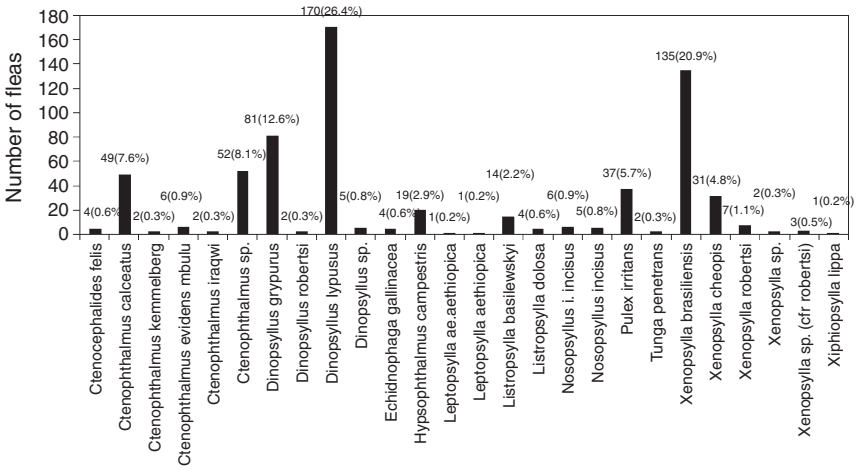

Fig. 2. Total number and percentage of total per species of fleas collected from rodent hosts in Karatu and Mbulu Districts, northern Tanzania.

are interconnected. Each point is a pie chart in itself, which indicates the habitats in which this species was found. So, for instance, if a point is 1/4 blue and 3/4 red, it was found in fields 25% of the times and in a domestic habitat 75% of the times.

There was a strong correlation (effect estimate = 2.7 ± 0.5, $r^2 = 0.71$, $P = 0.0001$) between sample size (the log-transformed number of individuals of a host species) and species richness (the number of different flea species found on a host species), which is common for species richness data. This indicates that the sample size would determine the diversity of fleas on a particular group of hosts. If more *Rattus* individuals were trapped, it is likely that more flea species would have been found. Because of this correlation, we used randomisation (bootstrapping) and rarefaction methods for statistical testing.

The values of the rarefaction curves served as a basis for comparing fleas species richness among host species. Figures 4 and 5 are the rarefaction curves for the different host species for sample sizes 0–116 (Fig. 4) and 0–10 (Fig. 5).

Table 1 shows the results for *P*-values to compare all combinations of host species in relation to their flea species richness. For example, the *P*-value for the hypothesis that the number of fleas on *Rhabdomys* sp. was larger or equal to the number of fleas on *M. natalensis* is 0.24, indicating that it is not rejected, and, therefore, the number of flea species is not assumed to be different. The *P*-value for the difference in the number of fleas between *L. flavopunctatus* and *P. delectorum* was 1, which means that *L. flavopunctatus* has significantly more flea species than has *P. dlectorum* (this was made clearer by reversing the hypothesis and, therefore, subtracting the *P*-value, 1, from 1, which gave a *P*-value of zero). These results were also clear from the rarefaction curves; the curve for *L. flavopunctatus* clearly lies higher than that of *P. delectorum*, indicating that it has more flea species.

Figures 6 and 7 show the rarefaction curves of different fleas species for sample sizes 0–100 and 0–10.

There were several flea species that were found on only one or two host species (ectoparasitic or free-living. Those that were found in a domestic environment, but not on a host, were *C. felis* ($n = 4$), *T. penetrans* ($N = 3$) and *E. gallinacea* ($n = 2$). Flea species found only on one host (*L. flavopunctatus*) were: *C. evidens mbulu* ($N = 6$), *X. lippa* ($n = 1$) and *C. kemmelberg* ($n = 2$).

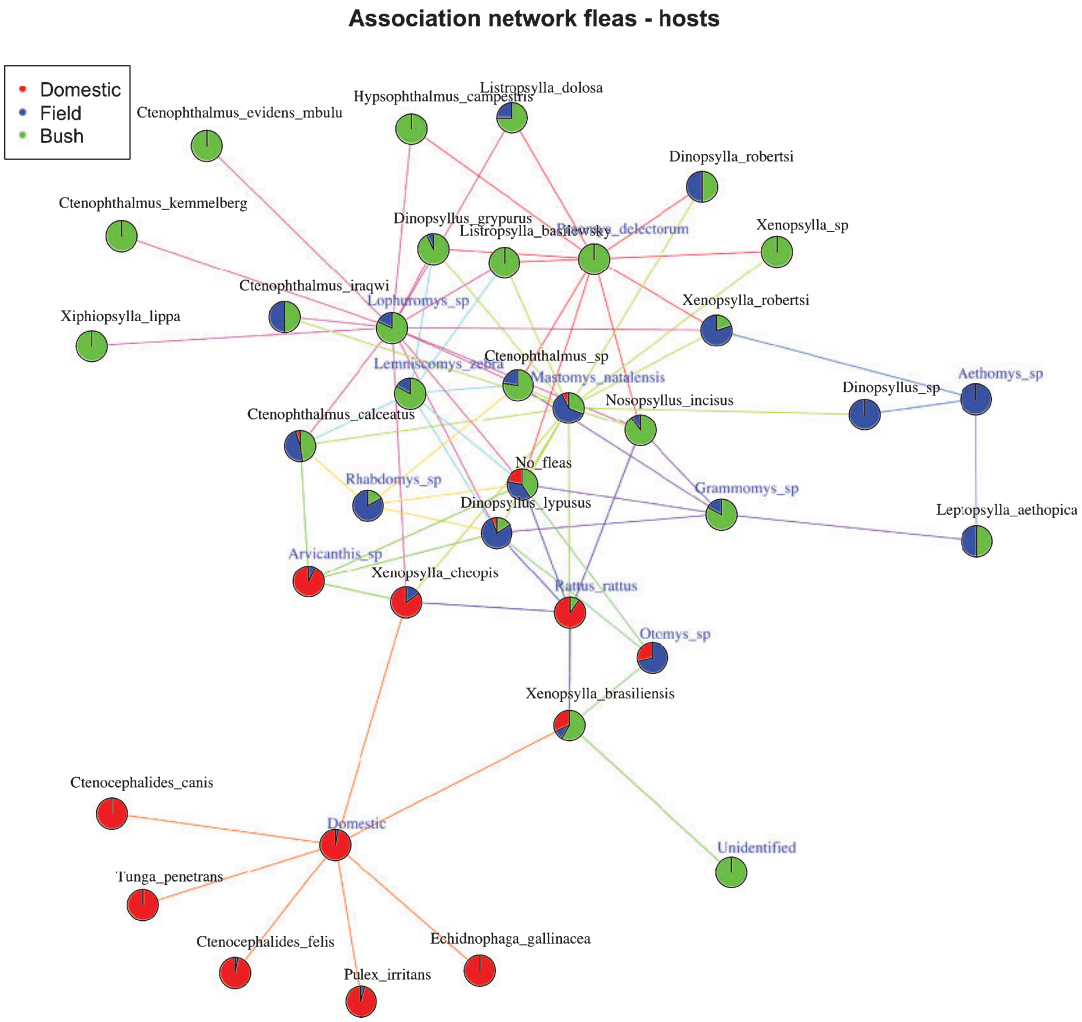

Fig. 3. Fleas, rodent and shrew host-association networks in different habitats in Mbulu district, northern Tanzania.

We carried out two-by-two comparisons of host species richness (Table 2). For each comparison, we calculated the *P*-values to test the hypothesis that the flea species in the first column have an equal or higher number of host species than do the flea species in the other columns. (e.g. *C. felis* vs *P. irritans* ($P = 0.75$); no evidence that *C. felis* does not have the same or a higher number of host species than does *P. irritans*; *P. irritans* vs *X. cheopis* ($P = 0$), so *P. irritans* has significantly fewer host species than *X. cheopis*). The results in Table 2 can be matched with the rarefaction plots (Figs 6, 7) where the different flea species can be compared. To determine whether two species differ significantly, the exact *P*-value can be found in Table 2.

We were interested to know whether host sex, reproducive age (adult or subadult) or habitat had any influence on the abundance or species richness of fleas. There was slightly significant correlation between sex of rodent host and flea abundance (the number of fleas on an individual; effect estimate $= 0.21 \pm 0.11$, $\chi^2 = 4.05$, d.f. $= 1$, $P = 0.44$), where flea abundance was higher on males. We also found that adults had more fleas than did subadults (effect estimate $= 0.38 \pm 0.12$, $\chi^2 = 10.18$, d.f. $= 1$, $P = 0.001$). The number of different flea species that was found on an individual did not correlate with any measured variable (species, habitat, sex, breeding status), with individuals having a mean number of flea species of 1.3 ± 0.1 (maximum $= 3$ different flea species on a host). There were significant differences in flea abundance between habitats ($\chi^2 = 57.692$, Df $= 7$, $P < 0.0001$), where the lowest flea abundance was found in forest and the highest inside houses.

Discussion

The current study contributes to understanding how fleas and rodent hosts are associated more specifically in a plague focus. We recorded 26 species of fleas in the plague outbreak foci in the Rift Valley, in comparison to the five species previously recorded (Msangi 1969; Kilonzo and Mhina 1983; Haule *et al.* 2013). In previous studies, Kilonzo and Mhina (1983) recorded seven rodent species in the plague outbreak areas in Mbulu District, compared with 13 species that were recorded in the current study. The present study has also provided evidence of a rich flea and rodent fauna in Mbulu and Karatu District plague foci. Rich flea and rodent fauna in plague outbreak foci was reported in the

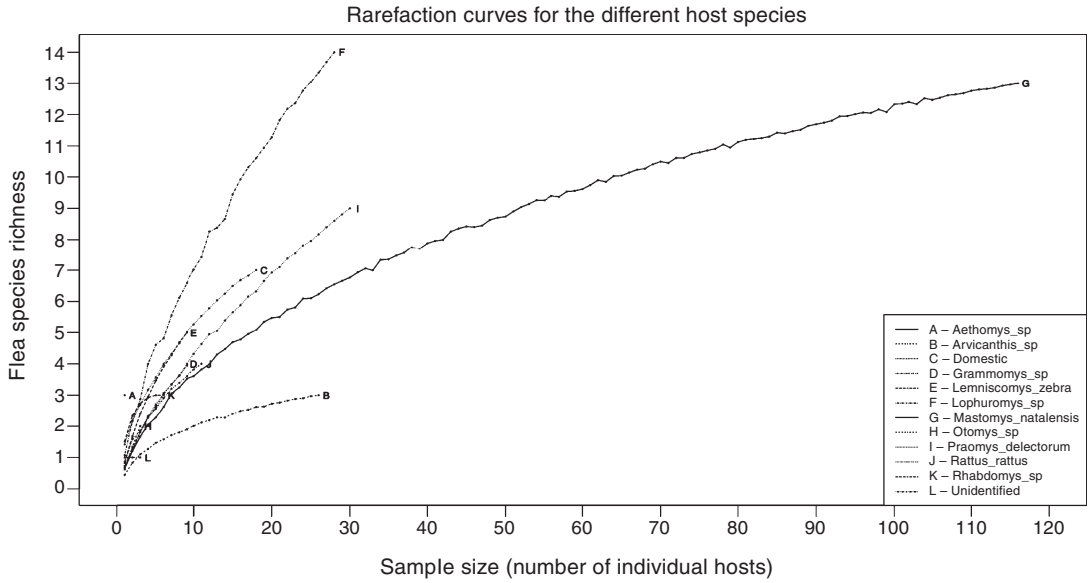

Fig. 4. Rarefaction curves for different host species (sample size 0–100).

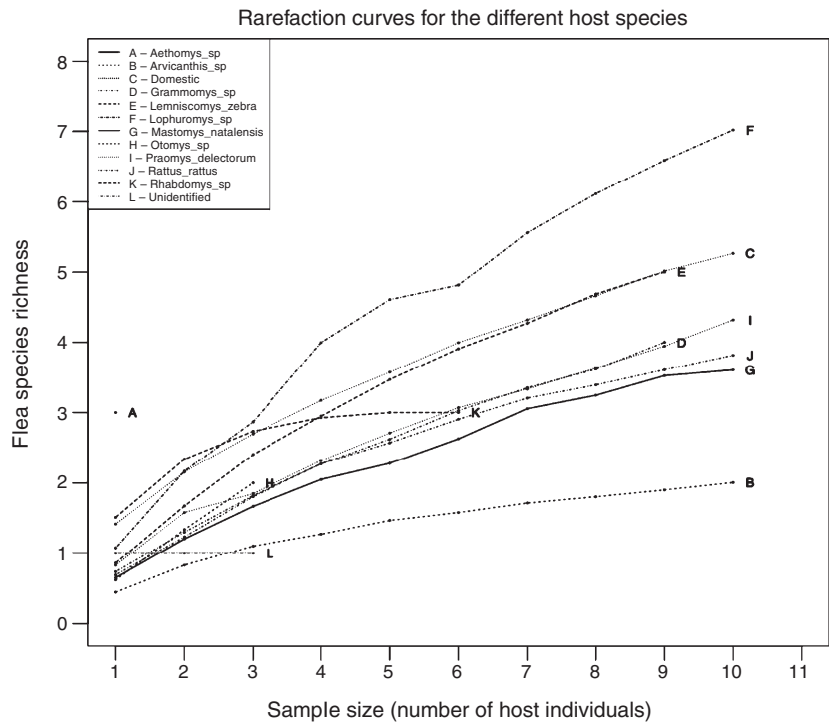

Fig. 5. Rarefaction curves for different host species (sample size 0–10).

Lushoto District in north-eastern Tanzania (Laudisoit *et al.* 2009*a*) and in earlier surveys of plague outbreaks in Mbulu District in northern Tanzania (Makundi *et al*. 2008).

Some flea species (e.g. *X. cheopis* and *X. brasiliensis*) were found within the domestic environment on hosts such as *R. rattus* and on semi-domestic species such as *A. niloticus* and *M. natalensis*, and some wild rats (field hosts), e.g. *L. flavopunctatus*, *Otomys* sp. and *Lemniscomys zebra.* This shows that these fleas are able to bridge the domestic and sylvatic rodents in an enzootic or epizootic cycle of infections. Studies conducted in Uganda observed similar association; these flea species were branded 'bridging vectors' (Amatre *et al*. 2009).

The results show a complex of ecological relationships between fleas and rodents, which is not unique to this plague

Table 1. A two-by-two comparisons for all combinations of host species and flea species richness

	Rhab	*Mast*	*Arvi*	*Ratt*	*Gramm*	*Loph*	*Prao*	*Unid*	*Otom*	*Lemn*	*Aeth*
Domestic	0.06	0.68	1.00	0.12	0.04	0.06	0.12	0.57	0.03	0.00	0.00
Rhabdomys sp. (*Rhab*)		0.24	0.76	0.06	0.35	0.07	0.15	1.00	0.00	0.00	0.00
Mastomys natalensis (*Mast*)			0.61	0.09	0.07	0.00	0.01	0.17	0.02	0.03	0.01
Arvicanthis sp. (*Arvi*)				0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
Rattus rattus (*Ratt*)					0.00	0.03	0.10	0.25	0.00	0.00	0.00
Grammomys sp. (*Gramm*)						0.27	0.27	0.34	0.19	0.00	0.00
Lophuromys sp. (*Loph*)							1.00	0.33	0.12	0.32	0.04
Praomys delectorum (*Prao*)								0.21	0.09	0.02	0.03
Unidentified (*Unid*)									0.00	0.00	0.00
Otomys sp. (*Otom*)										0.05	0.00
Lemniscomys zebra (*Lemn*)											0.00
Aeth = *Aethomys kaiseri*											

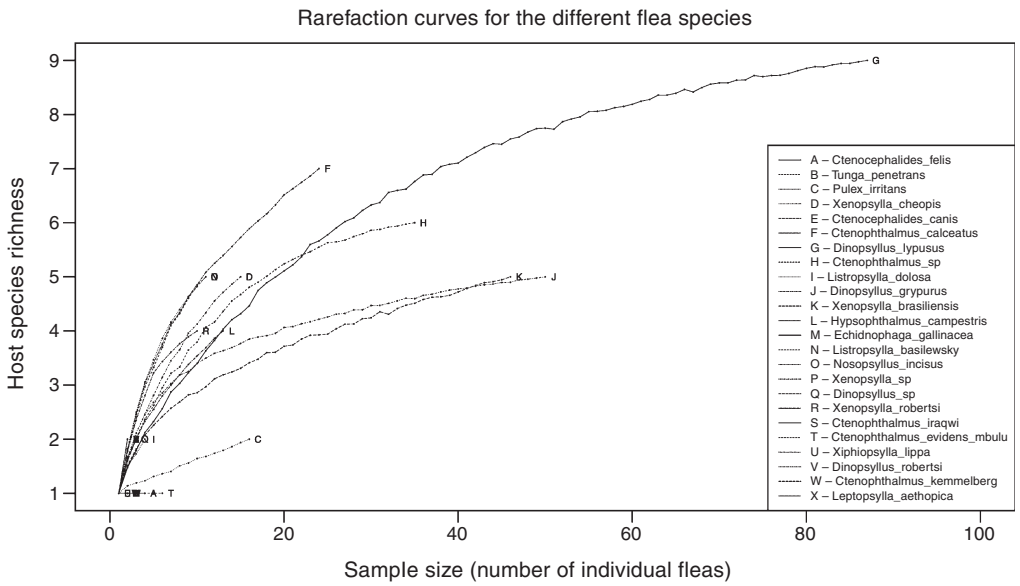

Fig. 6. Rarefaction curves of host species richness of different flea species (sample size 0–100).

focus; in the western Unites States, Gage and Kosoy (2005) reported at least 18 rodent species and 27 or more flea species being involved in enzootic plague cycles. In our study, both host and habitat overlaps were observed in different flea species. Lack of habitat specificity for flea species indicated that their distribution was greatly influenced by the distribution of hosts in different habitats. Apart from two species (*Mus* sp. and *Crocidura* spp.), all rodent species hosted more than one flea species, with the highest flea species richness found on *L. flavopunctatus*. The total prevalence of fleas on small mammals hosts was >60%.

Environmental factors are important in determining the abundance of fleas in different habitats or geographical regions (Durden and Hinckle 2009). However, it has been demonstrated that flea species richness is positively correlated with rodent species richness (Thiagarajan *et al*. 2008). Studies in the Negev Desert, Israel, indicated that environmental parameters (e.g. humidity, temperature and materials of the host's nest) affect the flea assemblages of a host (Krasnov *et al*. 1997). The parasite–host relationship was also influenced by habitat type (Krasnov *et al*. 1998). However, the linkages between flea communities, host communities and habitat types appear to be manifested differently in different geographical regions (Laudisoit *et al*. 2009*a*). Therefore, under different habitats, the diversity and abundance of hosts will determine the dynamics of interaction between fleas and rodents.

The network graph showed the pattern of interaction between rodents and fleas, and the multiple relationships in particular habitats were obvious. This enables understanding the relationship between flea species and rodent hosts, which can be applied to predict potential pathways of disease transmissions (Eames and Keeling 2002; Drewe 2010), or potential coinfections, with particular flea-borne agents such as *Y. pestis, Bartonella* sp. or *Rickettsia* sp. Flea hosts were not uniformly distributed. *L. flavopunctatus*, *P. delectorum* and *G. dolichurus* were predominantly found in the forest, whereas *A. niloticus* and *M. natalensis* were captured solely in fallow land and farmland. However, some flea species were commonly associated with them. Indeed, the network analysis showed that flea-sharing among hosts is far wider than hitherto reported. For example, Amatre *et al*. (2009) observed flea-sharing only among

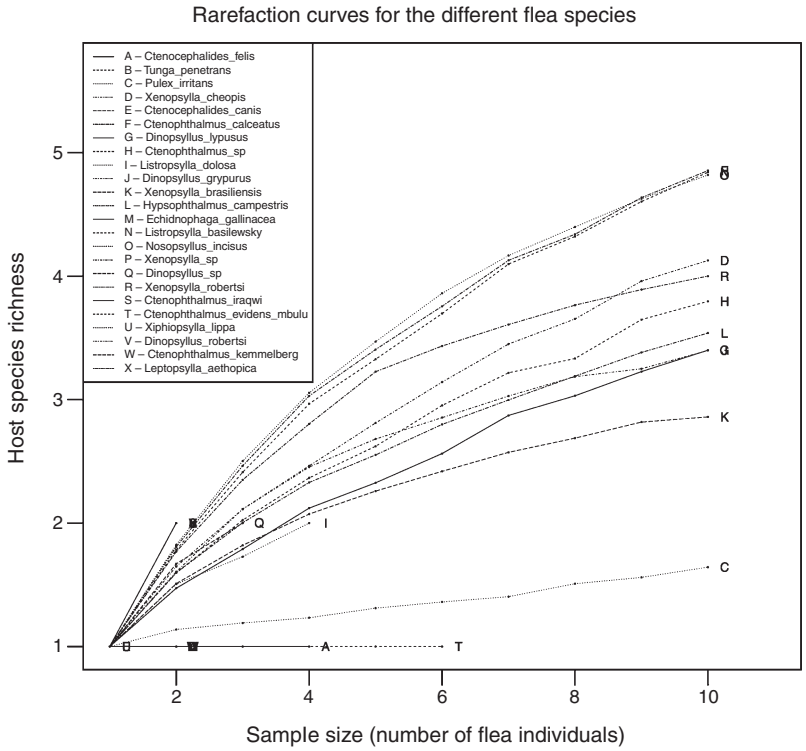

Fig. 7. Rarefaction curves of host species richness of different flea species (sample size 0–10).

few sylvatic rodents (*A. niloticus* and *M. natalensis* and with *R. rattus* and *A. niloticus* within the domestic environment).

Although, in our study, *R. rattus* and *A. niloticus* shared fleas, there is greater interaction and flea sharing among *P. delectorum*, *M. natalensis*, *A. niloticus*, *L. flavopunctatus* and *L. zebra*. Such interactions, especially involving several wild species of rodents, are necessary for maintenance of the enzootic cycle of plague (Wimsatt and Biggins 2009). The multiple networks shown in the current study suggest that an infectious host could easily infect other species through flea transfer, in particular because flea species are rarely host-specific and will feed on an available vertebrate host (Thomas 1996).

We found more fleas on adults than subadults, which is probably due to a typical positive correlation between weight/size and flea abundance ($\chi^2 = 4.1955$, d.f. = 1, $P = 0.040$). We could not establish any significant correlation between size and the number of flea species on an individual, and neither between sex and flea abundance. However, the significant correlation between breeding status and flea abundance indicated that by including breeding status in the model, the effect of weight is at least partly corrected for. Also, abundance analyses should take weight into account, because that is a known important predictor of flea abundance.

Our study has some implications for plague persistence and transmission. *Xenopsylla brasiliensis* and *D. lypusus* showed similarities in relation to their host associations. Considering that these two species are confirmed plague vectors, it is plausible to suggest that they are important vectors in enzootic plague cycle in the Rift Valley districts in northern Tanzania. *Xenopsylla brasiliensis* is known for its high vector efficiency and to share a broad spectrum of rodent hosts, and therefore is implicated to be primarily involved in plague transmission to humans in the peri-domestic and domestic areas (Gage and Kosoy 2005).

The complex flea–host associations within different habitats (forest, cultivated crop areas, fallow and domestic areas) most probably enable *Y. pestis* to be transmitted easily among hosts in the enzootic cycle, and when bactaeremia of the carrier hosts become sufficiently high, spread to humans during epizootics. Several host–flea complexes have been reported in regions of enzootic and epizootic plague (e.g. Davis *et al*. 2002; Gage and Kosoy 2005; Amatre *et al*. 2009, Foley and Foley 2010). Studies of flea–host associations in plague foci in Uganda showed that rodent hosts in domestic and sylvatic areas shared some common fleas (*D. lypusus*, *X. cheopis* and *X. brasiliensis*) that are capable of transmitting *Y. pestis* (Amatre *et al*. 2009) and a similar situation has been seen in Ituri, in the Democratic Republic of the Congo (A. Laudisoit, - pers. obs.). Our results also showed that these species of rodents are associated with wild and domestic rodents in the plague outbreak foci in the Rift Valley districts.

The results of the present study on how the flea species are connected to individual host species show that there are multiple channels for flea vectors to transmit *Y. pestis* among susceptible host species. It has been suggested that fleas living on hosts and in rodent burrows might significantly contribute to plague persistence (Wimsatt and Biggins 2009). Therefore, the presence of a large number of flea species in the study area that are connected to an equally large number of host species increases the potential for enzootic and epizootic cycles of plague to be maintained in the area. Studies in Uganda, for example, have

Table 2. A two-by-two comparisons of host species richness

For each comparison, we calculated the *P*-values to test the hypothesis that the flea species in the first column have an equal or higher number of host species than the flea species in the other columns

	Tp	*Pi*	*Xc*	*Cc*	*Cca*	*Dl*	*Cs*	*Ld*	*Dg*	*Xb*	*Hc*	*Eg*	*Lb*	*Ni*	*Xs*	*Ds*	*Xr*	*Ci*	*Cem*	*Xl*	*Dr*	*Ck*	*La*
Ctenocephalides felis	1.00	0.75	0.09	1.00	0.01	0.23	0.12	0.00	0.06	0.20	0.11	1.00	0.01	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	1.00	0.00
Tunga penetrans (*Tp*)		0.86	0.37	1.00	0.18	0.49	0.39	0.50	0.33	0.49	0.38	1.00	0.20	0.16	0.00	0.33	0.22	0.00	1.00	1.00	0.00	1.00	0.00
Pulex irritans (*Pi*)			0.00	1.00	0.00	0.03	0.00	0.24	0.00	0.10	0.00	1.00	0.00	0.00	0.13	0.18	0.00	0.12	1.00	1.00	0.12	1.00	0.14
Xenopsylla cheopis (*Xc*)				1.00	0.36	0.86	0.87	0.90	1.00	1.00	0.99	1.00	0.45	0.43	0.64	0.81	0.82	0.63	1.00	1.00	0.63	1.00	0.64
Ctenocephalides canis (*Cc*)					1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ctenophthalmus calceatus (*Cca*)						0.95	1.00	0.99	1.00	1.00	0.99	1.00	0.71	0.75	0.78	0.96	0.94	0.82	1.00	1.00	0.83	1.00	0.80
Dinopsyllus lypusus (*Dl*)							0.87	0.76	1.00	1.00	0.66	1.00	0.21	0.22	0.47	0.64	0.45	0.46	1.00	1.00	0.50	1.00	0.48
Ctenophthalmus sp. (*Cs*)								0.86	1.00	1.00	0.85	1.00	0.30	0.31	0.58	0.75	0.65	0.59	1.00	1.00	0.61	1.00	0.58
Listropsylla dolosa (*Ld*)									0.53	0.78	0.61	1.00	0.27	0.17	0.50	0.72	0.29	0.53	1.00	1.00	0.49	1.00	0.52
Dinopsyllus grypurus (*Dg*)										0.92	0.58	1.00	0.07	0.06	0.65	0.82	0.40	0.65	1.00	1.00	0.68	1.00	0.66
Xenopsylla brasiliensis (*Xb*)											0.31	1.00	0.02	0.02	0.50	0.69	0.21	0.52	1.00	1.00	0.49	1.00	0.49
Hypsophthalmus campestris (*Hc*)												1.00	0.02	0.02	0.50	0.69	0.21	0.52	1.00	1.00	0.49	1.00	0.49
Echidnophaga gallinacea (*Eg*)													0.00	0.00	0.57	0.80	0.57	0.62	1.00	1.00	0.64	1.00	0.60
Listropsylla basilewisky (*Lb*)													0.22	0.18	0.00	0.35	0.21	0.00	1.00	1.00	0.00	1.00	0.00
Nosopsyllus incisus (*Ni*)														1.00	0.76	0.93	1.00	0.77	1.00	1.00	0.76	1.00	0.77
Xenopsylla sp. (*Xs*)															0.83	0.97	1.00	0.80	1.00	1.00	0.81	1.00	0.81
Dinopsyllus sp. (*Ds*)																1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Xenopsylla robertsi (*Xr*)																	0.58	0.67	1.00	1.00	0.67	1.00	0.68
Ctenophthalmus iraqwi (*Ci*)																		0.79	1.00	1.00	0.79	1.00	0.78
Ctenophthalmus evidens mbulu (*Cem*)																			1.00	1.00	1.00	1.00	1.00
Xiphiopsylla lippa (*Xl*)																				1.00	0.00	1.00	0.00
Dinopsyllus robertsi (*Dr*)																					1.00	1.00	1.00
Ctenophthalmus kemmelberg (*Ck*)																						1.00	1.00

suggested that when there are multiple vectors interacting with rodent species, it increases the persistence of plague in an active focus (Eisen *et al*. 2012).

Available evidence also indicates that the transmission of plague in sylvatic reservoirs is almost exclusively undertaken by fleabite (Bearden and Brubaker 2010), which suggests that the more complex the degree of association between hosts and fleas, the greater the potential for circulating the bacterium among a community of hosts. However, there are some plague-outbreak scenarios, such as in Madagascar, which is the most affected country in the world, where outbreaks involve only *R. rattus* and two flea species, namely, *X. cheopis* and *Synopsyllus fonquerniei* (Rahelinirina *et al*. 2010). The plague epidemics in Madagascar are not typical of established outbreak models in other parts of Africa. This is attributed to large populations of rodents and fleas, the latter being resistant to first-line insecticides, and is also exacerbated by anthropological factors, mainly increasing human population density, substandard housing infested with large numbers of rodents and fleas, and insufficient health-care industry (Winter 2014).

In previous studies in the Lushoto District, where plague outbreaks were recorded between 1980 and 2004 (Davis *et al*. 2006), differences in flea species diversity and abundance between plague-free and plague-endemic villages were reported, with higher diversity and abundance of fleas in the outbreak villages (Laudisoit *et al*. 2009*b*). Similar observations have been made in other foci in East Africa, for which high flea diversity was strongly associated with plague outbreaks (Eisen *et al*. 2012). It is, therefore, plausible to suggest that the high species richness of fleas and rodents in the Rift Valley districts contribute to maintenance and persistence of plague for which sporadic cases in humans have been recurring since 2008.

Although the study showed a complex association and interaction between the potential vector and hosts, it did not shed any light on vector efficiency and reservoir potential of the hosts; laboratory studies accounting for level of parasitism in relation to immune status of the hosts are required to establish both.

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