Quantitative inventories of ants from India: gaps, prospects and recommendations

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India is regarded as one of the highly diverse but data-deficient regions in terms of ant diversity. Despite a number of studies, patterns in diversity of ants in India are still unclear. Through a review of recent Indian studies based on samples of ground dwelling ants, we highlight reasons that may underestimate diversity and hinder comparisons. This study shows that recent developments in sampling ant assemblages and analysis of the data have not sufficiently rooted in India. In addition, several geographic areas are still under-explored and need further attention. Therefore, it is important that future inventories adapt methods that facilitate comparison of data. In this regard, we provide results that reiterate some of the important developments in designing effective inventories for ants. The appraisal of data through such methods is expected to improve the knowledge about Indian ant fauna and its distribution.

Keywords: Ant sampling, ALL-protocol, Arunachal Pradesh, inventory of ants.

ANTS are increasingly used as focal taxon for ecological and conservation studies, mostly due to their biological properties. They are present in diverse habitats from cold temperates to warm tropics, from leaf litter to tree canopies, and even in urban dwellings. Ants play a key role in the ecosystem as predators, pollinators, seed dispersers and as symbiotic partners. Species richness and composition of ants change rapidly across gradients of climate, habitats and life histories[1]. Their numeric as well as functional responses to environment, along with more or less sedentary nesting, and almost ubiquitous distribution make ants excellent candidates for studying the patterns and processes of biodiversity[2–4].

Since sampling subterranean or canopy ant assemblages is too cumbersome and the methods not adequately standardized, the ground dwelling ants are the convenient choice for ecological studies. Considerable developments have taken place recently in the methods to inventory ground ant assemblages[5]. Active search, bait traps and pitfall traps are some of the most common methods to study ants. Active searching has the advantage of versatility. It can be used in any habitat and can be adapted for quantitative sampling, through using time-constrained search. However, the data collected is very likely affected by experience and skill of the researcher. Baited traps are easy to use, and the best method for recording behaviour in field but it is not a suitable method for inventory, as the composition of ants will depend on the baits used. Pitfall traps are one of the most widely used methods to sample ants, and they perform well in open areas with small amounts of leaf litter. The best known method for sampling leaf litter ants is the 'Winkler leaf litter extraction' and this technique is known to outperform the conventional techniques like 'pitfall traps'[6,7].

Agosti and Alonso[8] have proposed 'ants of leaf litter (ALL)', a standardized protocol for inventory of ants to obtain quantitative estimates of ant species richness to allow comparison across different studies. Inventories generated through similar replicable protocols have been effectively used[9] to develop methods to estimate species richness and to compare the efficiency of estimators. Such results have been of general importance to quantitative ecology. Inventories with standardized protocols and sampling units have facilitated diversity comparisons on large scales[10]. However, these developments are not sufficiently rooted in India. Large scale studies on estimates of local diversity[11] and regional diversity[12] highlight India as a data-deficient region. Particularly, there seem to be very few studies that estimate diversity of ants and study spatial variation in diversity.

In this paper, we focus on the knowledge gap about local diversity and its patterns. We explore reasons for this knowledge gap through a synthesis of recent ecological studies from India that use clearly defined methods to sample ants. We also carried out a field experiment in the Eastern Himalayas to test the efficacy of the ALL protocol[8] for inventorying ants for ecological studies in the Indian tropical forests. We ask two questions 'how efficiencies of Winklers and pitfall traps compare with each other?' and 'what is the adequate sample size for a nearly complete inventory in the Eastern Himalaya?'

Methods

Review of quantitative sampling studies

We conducted a literature search, using 'Google Scholar' (with search string 'India' 'Indian' 'ant' 'ants'), for

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Indian studies on ants that use clearly described inventory protocols. This search string resulted in more than twenty thousand search results. From these, we excluded studies on behavioural and chemical aspects or any species-specific studies based on the titles in the search list. This eliminated most of the studies. To filter the studies further we broadly followed the guidelines originally proposed by Dunn *et al.*[11]. This meant that we only considered studies that were using samples of ant communities from one or more sites and excluded publications based solely on secondary data or mere species checklists. We continued the search until we did not encounter any new studies for 200 consecutive search results, a technique similar to reaching asymptote of species accumulation curve. In addition, we also manually examined the large list of references provided in the recent updated checklist of Indian ants[13]. We reviewed these studies based on, observed and estimated species richness (if provided), number of replicates used, and intensity of sampling within each replicate. We also used the metadata in the papers such as the year of publication and size of sampling units if reported, to explore whether there are any patterns across years.

Field experiment in eastern Himalaya

We carried out a sampling experiment in the Eaglenest Wildlife Sanctuary (92.40E, 27.066N) and the Pakke Tiger Reserve (96.66E, 27.04N), part of Eastern Himalaya in the western part of Arunachal Pradesh. The field experiment was based on the premise that inventories carried out using standardized methods yield datasets for addressing ecological questions and are more resourceful than checklists. Therefore, results generated from surveys should be an adequate representation of the actual assemblages, in order to be useful for further analysis. However, there was little prior information available about ant fauna or efficacy of trapping methods from the study area that could guide choice of methods or amount of sampling effort. Therefore, we first carried out a pilot survey with a modest sampling effort. We followed this with a more extensive sampling.

During the pilot survey, we used 10 pitfall traps and 10 Winkler extractors at an interval of 10 m on a 100 m transect at 1200 m elevation. Plastic glasses with 8 cm diameter were used as pitfall traps. The traps were buried with mouth of the trap just below the ground level. We used 70% alcohol as fixative in the traps. Winklers are designed to sample insects from the leaf-litter. To facilitate the process, leaf-litter is first sifted using a sieve (0.8 mm). The sifted litter is then placed in mesh bags of the same size, which are suspended inside the Winkler bags. Insects that crawl out of the bags due to disturbance and changes in microhabitat fall into a receptacle. We made the Winklers as per the dimensions given by Bestelmeyer *et al.*[14] and the collection was carried out according to the protocol outlined by Agosti and Alonso[8].

Using the pilot survey results, we derived estimates of species richness[15] and the corresponding sampling effort[16] necessary for an almost complete inventory. We then carried out additional sampling based on the estimated richness and sampling effort. This subsequent sampling was carried out at two different elevations – 200 m and 600 m. At each elevation we sampled along four different transects with the same protocol used during the pilot sampling.

All the specimens collected from each single trap were sorted to morpho-species. Genera were identified from keys prepared by Bolton[17], wherever possible species name was assigned using keys given by Bingham[18] or recent monographs available on the respective genera. Species names were updated and checked for synonyms using hymenoptera name server (http://osuc.biosci.ohio-state.edu/).

Analysis

To check the efficacy of using the species accumulation curves for predicting asymptote in cumulative richness and the corresponding sample size, we compared 'Michaelis-Menten' equation[9] – a commonly used asymptotic model and a non-asymptotic model proposed by Soberon[19]. If the observed part of the accumulation curve is adequate to predict the asymptote in cumulative richness then 'Michaelis-Menten' equation should perform better than non-asymptotic model. We also estimated the necessary sampling effort needed to achieve 'Chao 2' (ref. 20) – a commonly used non-parametric estimate of species richness, using methods developed by Chao *et al.*[16]. We used occurrence-based (number of traps in which a species is recorded) rarefaction curves as well as sample-based curves[21] to show differences in rates of species accumulation between the two trapping methods – pitfall traps and Winklers. Proximity of a trap to ant nests or trails can inflate the number of individuals collected and therefore instead of abundances we used occurrences[22,23].

We also checked the efficiency of using an additional collection technique by comparing species accumulation rates for the data subsets for each trapping method. We treated each trap as separate sampling unit and compared the accumulation rates between subsets after retaining either of the traps. We also performed similar analysis after pooling the individual traps into trapping stations. We compared these results with another study which used the same methods of comparison[6].

Results

Quantitative inventories in India

We found 18 publications that fit our criteria (see Supplementary Table 1). Out of these, six studies used inventory designs based on the ALL protocol and used Winklers to sample leaf-litter assemblages. Most publications (eight out of eighteen) were from the Western Ghats. Pitfall trap was the most commonly used method, followed by hand collection and Winklers (see Supplementary Table 1).

Compared to the Western Ghats, studies from the Himalaya are fewer and more recent. Three studies from the Western Himalaya have used designs similar to the ALL protocol[24,25]. Apart from these two regions (Southern Western Ghats and Western Himalaya), other biogeographic regions of India like the Eastern Ghats, Satpura ranges and the Eastern Himalaya have hardly received any attention so far, except for the single study from Eastern India[26] and another from Meghalaya[27].

There is an apparent increase in the number of quantitative studies per year in recent past (Figure 1). Seven studies out of the eighteen had reported the area within which traps were placed or hand collection was carried out (Supplementary Table 1). Based on the descriptions of sampling designs for these studies, the size of the smallest unit used for sampling appears to have reduced (Figure 2) across the years and the number of such units used to report the diversity estimates has increased (Figure 3).

Most studies (ten out of eighteen) used counts of worker ants in traps while seven studies used incidence (frequency of occurrence) data. Eight other studies used species richness estimators but one of these[28] has used diverse set of methods (Winklers, pitfall traps, bait traps, among others) as replicates to generate the estimates.

Field experiment of sampling ants from eastern Himalaya

We collected 35 species during the pilot sampling (at 1200 m) across 103 occurrences. In the final sampling,

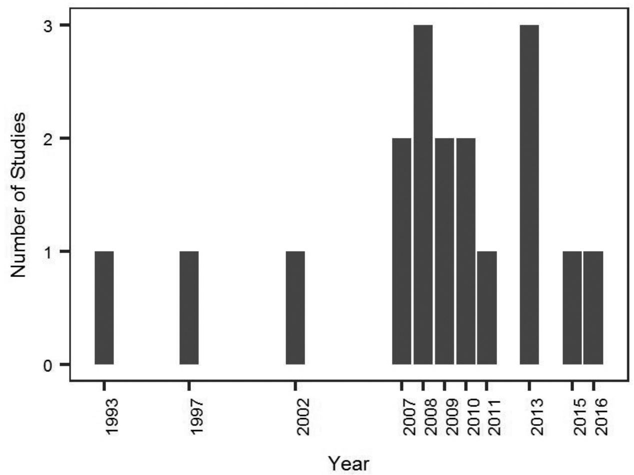

Figure 1. Number of studies with standardized samples of ants published from India across years.

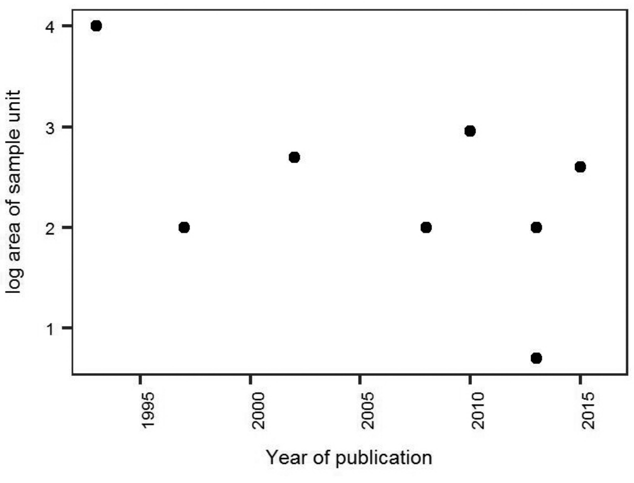

Figure 2. Area of the sampling unit used in the study. Only studies that used plots or quadrates were considered.

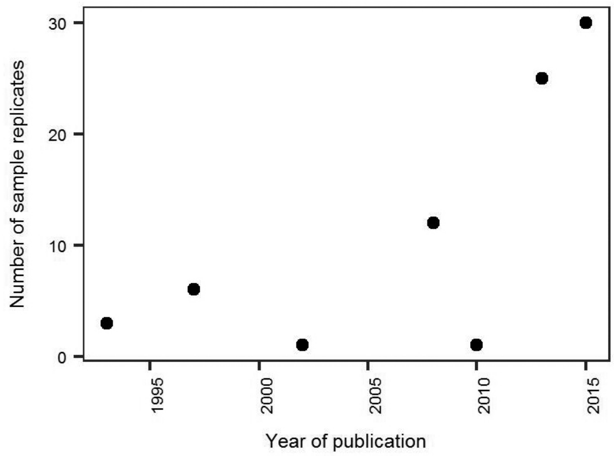

Figure 3. Number of sampling units pooled to report the estimates of diversity (using the same subset of data as in Figure 2).

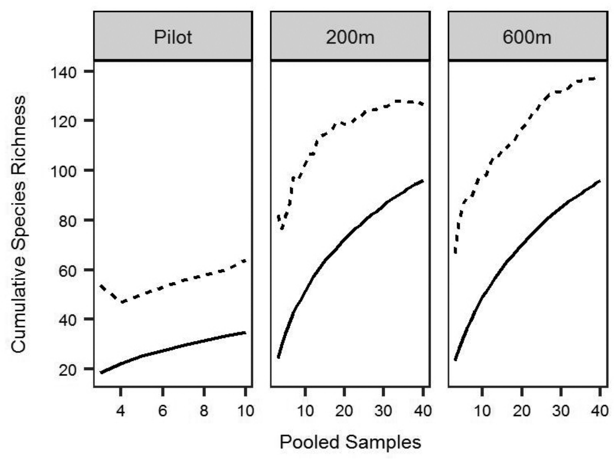

Figure 4. Rarefaction curves for observed and estimated species richness of the pilot and final samples.

Table 1. Parameter estimates for models fitted to the accumulation curve and estimated sampling effort for the cho 2 estimated richness

Sample	Michales–Menten (V_m, K, Rsq)	Log model (z, a, Rsq)	Observed richness	Chao 2 estimate	Estimated sampling effort
Pilot	55.5 ± 0.6, 10.9 ± 0.6, 0.96	0.05 ± 0.2*10^{-2}, 5.8 ± 0.02, 0.99	35	70	103
600 m	133.1 ± 1.14, 1.5 ± 0.8, 0.99	0.02 ± 0.01*10^{-2}, 4.04 ± 0.02, 0.99	96	135	285
200 m	129.8 ± 1.1, 28.7 ± 0.6, 0.99	0.02 ± 0.01*10^{-2}, 5.8 ± 0.03, 0.99	96	126	202

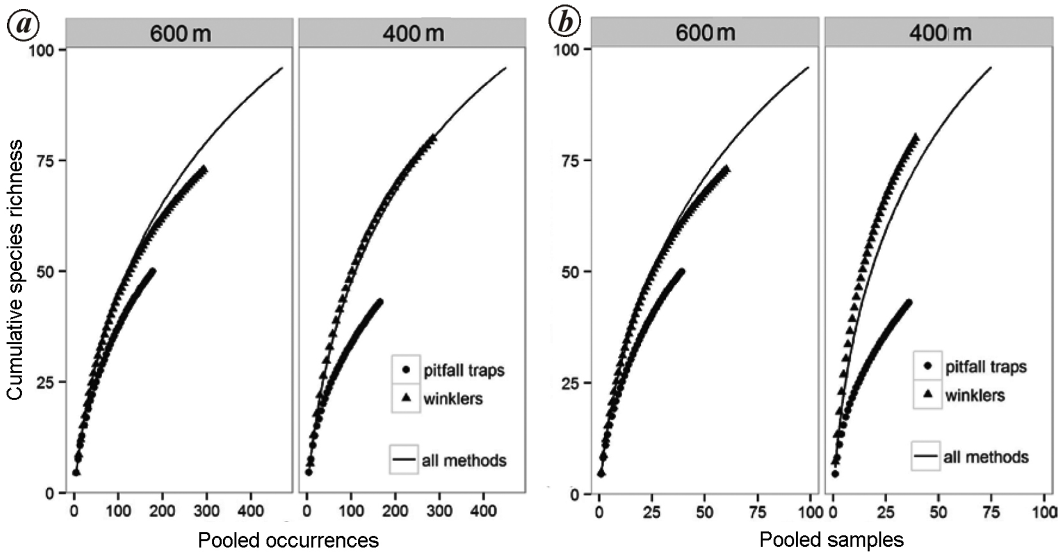

Figure 5. Species accumulation curves for each trapping method by pooling (***a***) number of occurrences and (***b***) number of traps.

we collected 96 species at 200 m across 405 occurrences (2019 individuals) and 96 species across 413 occurrences (3342 individuals) at 600 m. Visual examination of species accumulation curves showed decrease in rate of species accumulation but no clear asymptote was reached, while the estimated richness did appear to stabilize at higher sample sizes (Figure 4). 'Michaelis–Menten' model as well as the non-asymptotic log model fitted the data equally well (Table 1). For data obtained during pilot sampling, the estimated effort needed to achieve nearly complete inventory was 4.6 times greater (93 traps). For the final sample this deficit was 2.3–2.8 times the present effort (note that these multipliers apply to a larger sample size of 80 traps, therefore the projected sample size is much higher even if the index is lower; Table 1).

Winklers collected species at much higher rate than pitfall traps. The difference between the methods was consistent even after plotting the accumulation curves based on cumulative occurrences rather than cumulative number of samples (Figure 5). Therefore the difference in number of species recorded by each method is not an artefact of recording more occurrences in Winklers (for more explanation on accumulation curves see ref. 21). Further, after rarefying for the number of occurrences, trapping stations with and without pitfall traps had comparable rates of species accumulation (Figure 6). This shows that pitfall traps contribute little to cumulative increment in species richness and most species captured in pitfall traps were subsets of species captured in Winklers.

Discussion

Complete enumeration of ant communities is not possible with rapid surveys and is difficult with large and long-term inventories. The results from our sampling effort estimation indicate that an effort of about 200 trapping units can only bring out a nearly complete inventory (95% of the estimated richness) at local scales (Table 1). Such high effort is possible during a taxonomic inventory but may be impractical for ecological studies, where several spatial and temporal replicates may be required for making reliable inferences. Therefore, complete inventories in any large landscape like the Eastern Himalaya are not possible through single sampling expedition. An easy way to address this is to depend on secondary data generated through repeated sampling from different studies. Such samples can be of greater scientific value if the sampling follows standardized and replicable protocols. At present our abilities to generate such inventories using multiple studies or use the data to compare estimates of diversity are limited primarily due to scarcity of data, but

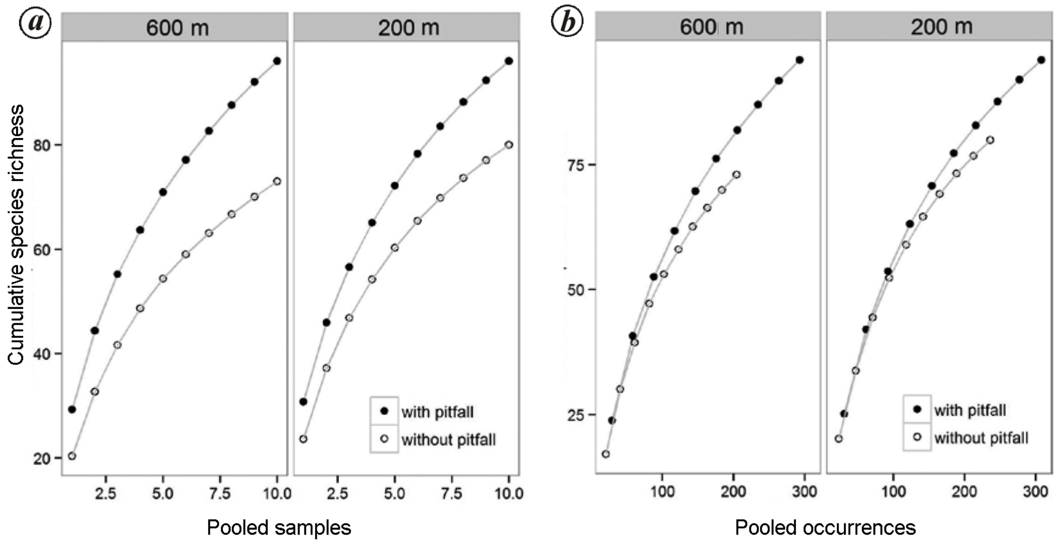

Figure 6. Species accumulation curves for trapping stations with and without pitfall traps by pooling (***a***) number of trapping stations and (***b***) number of occurrences.

equally so due to incompatibility of sampling protocols and the results reported.

An extensive literature search revealed only eighteen publications based on samples of ground ant assemblages with a strong bias towards the Western Ghats (Supplementary Table 1). The oldest study in the review was published in 1993. This shows that rate of publications is almost less than one study per year. This is due to a lack of attention towards quantitative studies on ant assemblages. Thus there is a need for more studies that follow standardized protocols to facilitate comparisons across sites and make better regional level assessments possible. This would be an important step towards addressing existing knowledge gaps in ant diversity studies in India. Such studies can provide valuable baseline information towards the effects of local disturbances like land-use change and for studies on global processes like climate change.

The studies we reviewed showed two interesting trends – decrease in the area of sampling unit and increase in the number of sampling units used across the years. These two trends together suggested that recent inventory designs contained more information about sample heterogeneity, compared to older studies where each datum was sampled at much larger area. Statistical analysis of these trends is not possible as they are from an even smaller subset of the data that use plot-based designs.

The studies we reviewed, used between one and five trapping methods for sampling ants. A general justification for using multiple methods is that each technique has an unique set of target species and a combination of techniques will increase the inventory efficiency. However, it is difficult to separate the effects of sample size increase from addition of different collection methods, such as the difference between using 10 pitfall traps and 10 baits versus using 20 pitfall traps[29]. More importantly, observed species richness of samples collected with different collection techniques are not directly comparable. Samples can be comparable across studies if similar methods are used and rarefied estimates are reported, to account for differences in sample sizes.

Another observation from the review is that the use of Winklers has not been very common. Winkler leaf-litter extractors are sampling devices specially designed to sample ants from the leaf-litter, which were first described by Holdhaus[30]. The technique was later adapted by several other researchers who made detailed comments about operating Winklers and efficiency of this method[6,7,30]. These studies equivocally confirm that Winklers outperform other trapping methods when sampling leaf-litter ant assemblages. In the studies reviewed here, only three inventories used Winklers, one in the evergreen forests of southern Western Ghats and the other two from the subtropical dry deciduous forests in Himalayas. Without the use of Winklers, leaf-litter ant assemblages will remain poorly sampled and regional richness is very likely to be underestimated.

The number of species reported using any method is invariably a negatively biased estimate of actual species richness. Estimators of species richness based on observed commonness and rarity are therefore important[31]. In our review, among the few studies that did report sample or aggregated estimates of species richness, two were using counts of worker ants as measure of rarity. Using worker abundance was common for reporting other measures of diversity as well. Problems with using raw counts of workers in traps have been mentioned several times in the literature[22,32,33] so we will not discuss it in detail here.

From the above observations, we conclude that, there are important knowledge gaps which the future inventories should address. The most serious are the dearth of data and inventory effort. Most of India is largely data-deficient, but the Western Ghats has received relatively more attention. There is a recent effort building up in the Western Himalaya, while the Eastern Himalaya is almost completely data-deficient region. Large differences in sampling protocols (Supplementary Table 1) and inappropriate use of the data are also important hurdles while comparing the species richness across studies. In this regard, we clearly describe the inventory protocol used for a field experiment to inventory ants in the Eastern Himalaya. The protocol was designed based on some of the earlier findings on inventory design[6,8]. We also report three key features of the results namely the estimated richness, estimated sample size to achieve a nearly complete inventory and accumulation curves. We hope this information will be useful in designing future inventories in the region.

For the field experiment we used a set of ten Winklers and pitfall traps on four separate transects, unlike the 25 traps recommended in the 'ALL protocol'. As all the replicates in the field experiment are in the same habitat and in the same continuous forest patch, we pooled the replicates together and performed the analysis on all 40 traps of each method. Therefore, our sample sizes are comparable to other studies that compared the efficiency of collection methods[6,7,34].

The two models used – asymptotic and non-asymptotic, describe the accumulation curves equally well (Table 1) and hence, it is not possible to find the hypothetical asymptotic function, which may predict the real asymptote using the available data. We therefore used non-parametric estimator (Chao 2) for species richness and the estimated corresponding sample sizes (Table 1). Rates of accumulation of the Chao 2 estimator did decrease towards higher sample sizes and in only one of the sites and it was approaching asymptote (Figure 4). This indicated that our sample size was not sufficient for a complete inventory but enough to generate a robust estimate of species richness. The estimates reported here are likely to be negatively biased if compared to an exhaustive inventory of the same area with much more sampling effort, as multiple regions of stability in estimated richness may appear in very large samples[9]. Hence, the results from our method represent readily comparable estimates of species richness from a rapid survey rather than exhaustive inventory.

The results of our sampling experiment are similar to the example from Madagascar[6] where pitfall trap collections were subset of Winkler collections. The difference between rates of species accumulation with Winklers and pitfall traps was more prominent in Madagascar study where the Winkler collections closely approximated the total collections using both the methods. Compared to these results, the two methods are relatively less different in our study, possibly due to larger diameter of the pitfall traps used (1.8 cm test tubes in the Madagascar study versus 8 cm plastic cups in the present study). Other studies that compared Winkler efficiencies with other methods have also found Winklers to be more efficient[7,34].

While the ALL protocol includes both Winklers and pitfall traps, Winklers are more efficient in leaf-litter habitats and therefore more preferable in the evergreen forests of Eastern Himalaya. It may be argued that Winklers collect more individuals than pitfall traps and therefore increase the workload during curation. Since they, accumulate more species compared to pitfall traps for every set of individuals sampled (Figure 5), the increase in the laboratory effort is still efficient. Moreover, Winkler samples are much cleaner compared to pitfall traps, as the latter tend to accumulate a lot of dirt and mud if left open for too long, which also slows down the curation process. In addition, pitfall traps generally need to be kept for extended duration in field to be effective, which makes them susceptible to sudden events like rainfall or damage by animals. Comparatively, Winklers collect a snapshot of litter from the field and are generally stored within the relative safety of field station or a bush camp. Therefore, they are more efficient as well as dependable.

We would like to mention that some of the oldest documentations of ants, dating back to 1854, were made in India[17,18,35]. Since then, through meticulously assembled checklists, taxonomic analyses and descriptions of new species, the number of ants known from India is now 829 compared to 498 in the fauna of British India published in 1903 (ref. 18). While such work is remarkable in its own right, it does little to shed light on mechanisms limiting and shaping ant communities. Quantitative data on spatial variation of diversity is a key tool for understanding such questions. With this review and description of our field protocol, we wish to highlight the need for increased efforts towards ant inventories and at the same time advocate the use of clearly defined protocols so that the inventories are comparable and the data can be available for ecological analysis.

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