**Supplementary Material 1:** Antsampling protocol from the School of Ants citizen science project.

Make Your Own Collection for the School of Ants

MATERIALS

* This page, printed out
* 8 white 3x5" index cards   
  (or similar sized paper)
* 1 pen or pencil
* 2 Pecan Sandies cookies\*
* 2 large zip-lock bags (1-gallon)
* 1 envelope for submitting your kit by US mail, plus postage

*\*Allergy warning: contains nuts!*

|  |  |
| --- | --- |
| **COLLECTION DATA** | |
| Collector Name | Address where ants were collected |
| Weather (e.g. sunny, cloudy, rainy) | Temperature (°F) |
| Collection Date | Time of collection |
| Habitat type: GREEN  (e.g. lawn, forest, garden) | Detailed habitat: GREEN  (e.g. base of tree, in grassy yard) |
| Habitat type: PAVED  (e.g. sidewalk, driveway, playground) | Detailed habitat: PAVED  (e.g. gravel driveway, street corner) |
| **CONFIRMATION CODE:** (Issued after you enter collection data online) | |

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Thanks for joining our investigation of the ants that live around us! The School of Ants is mapping ant species in cities and towns, starting in your backyard or school. Your samples help us to understand local biodiversity, document native and introduced species, and will help us track the effects of climate change and urbanization. To get started, follow these **5 easy steps** to collect and submit a sample. *After we receive and identify your ants, we will alert you by email that your ants are mapped!*

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**Step 1: GREEN SPACE.** Take 4 notecards and 1 cookietoa green outdoor space (lawn, garden or forest), preferably on a warm sunny day. Write the word GREEN on each of the cards and place them on the ground about 1 foot apart. Place a rock on the cards if it is windy! Break the cookie into 4 pieces and crumble each on top of a card. Be sure the edges of the cards touch the ground so ants can easily discover your delicious crumbs. *Record your collection information on this sheet.*

**Step 2: PAVED SPACE.** Repeat step 1, but choose a nearby site that has paved surfaces (e.g. sidewalk, driveway, playground) and write PAVED on each card. R*ecord your collection information on this sheet.*

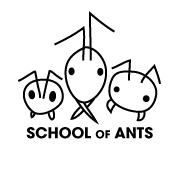
**Step 3: COLLECT.** One hour after you set out your cookies, it is time to collect the ants! Pick up each card that says GREEN and quickly pour the crumbs, ants and card into a large zip-lock bag (*Note: some ants can sting, so be careful*. *Use extra caution if you are allergic to bee or wasp stings.*) Seal the bag and write GREEN on the outside! Now repeat with the PAVED samples in a *separate* bag. Both the cards and the label on the bag should show where your ants were collected (Green vs. Paved). To preserve your samples for research and prevent them from decomposing, place both bags into the freezer overnight. This is a humane way to sacrifice ants.   
*\*Suggestion: leave a bit of air in each bag to cushion the ants so they won’t get crushed in the mail!*

**Step 4: REGISTER.** Register your kit at [www.schoolofants.org](http://www.schoolofants.org). You will need to make a username and password. Follow the prompts to enter your data and you will receive your **Confirmation Code.** *Write this Confirmation Code in the data table on this sheet.*

**Step 5: SUBMIT.** Submit your sample after completing this checklist.

Your package should include:  
2 large bags labeled GREEN or PAVED, each with 4 cards and some crumbs and hopefully full of ants, frozen overnight.  
This sheet, with the CONFIRMATION CODE you received after entering your collection data online, printed clearly above.  
 Send your kit to

The School of Ants Team, Dept. of Entomology / Nematology  
970 Natural Area Dr., University of Florida, Box 110620  
Gainesville, FL 32611 USA. *NOTE that you are responsible for postage!*



From the whole School of Ants Team, Thanks for participating!

**Supplementary Material 2:** Specimen data associated with all *Tetramorium* *immigrans* individuals used in this study, including *COI* Genbank accession numbers, ddRADseq SRA accession numbers, the State in which it was collected, and the geographic region it was assigned to.

|  |  |  |  |
| --- | --- | --- | --- |
| ***COI* Accession #** | **SRA Accession #** | **State** | **Region** |
| KT339872 | SAMN11519431 | WA | Western |
| KT339873 | SAMN11519405 | NY | Northeast |
| KT339874 | SAMN11519395 | PA | Northeast |
| KT339875 | SAMN11519398 | PA | Northeast |
| KT339876 | N/A | MD | Northeast |
| KT339877 | N/A | IL | Midwest |
| KT339878 | SAMN11519381 | IL | Midwest |
| KT339879 | SAMN11519385 | IL | Midwest |
| KT339880 | SAMN11519386 | IL | Midwest |
| KT339881 | SAMN11519389 | IL | Midwest |
| KT339882 | SAMN11519392 | IL | Midwest |
| KT339883 | SAMN11519432 | MT | Western |
| KT339884 | N/A | MA | Northeast |
| KT339885 | SAMN11519402 | CT | Northeast |
| KT339886 | SAMN11519403 | NY | Northeast |
| KT339887 | SAMN11519406 | NY | Northeast |
| KT339888 | SAMN11519407 | NY | Northeast |
| KT339889 | SAMN11519408 | NY | Northeast |
| KT339890 | SAMN11519409 | NY | Northeast |
| KT339891 | SAMN11519397 | NJ | Northeast |
| KT339892 | SAMN11519404 | NJ | Northeast |
| KT339893 | SAMN11519443 | NJ | Northeast |
| KT339894 | SAMN11519442 | NJ | Northeast |
| KT339895 | SAMN11519394 | PA | Northeast |
| KT339896 | SAMN11519396 | PA | Northeast |
| KT339897 | SAMN11519401 | PA | Northeast |
| KT339898 | SAMN11519410 | PA | Northeast |
| KT339899 | SAMN11519441 | MD | Northeast |
| KT339900 | SAMN11519400 | DC | Mid-Atlantic |
| KT339901 | SAMN11519446 | VA | Mid-Atlantic |
| KT339902 | SAMN11519399 | VA | Mid-Atlantic |
| KT339903 | SAMN11519415 | TN | Mid-Atlantic |
| KT339904 | SAMN11519412 | NC | Mid-Atlantic |
| KT339905 | SAMN11519413 | NC | Mid-Atlantic |
| KT339906 | SAMN11519414 | NC | Mid-Atlantic |
| KT339907 | SAMN11519416 | NC | Mid-Atlantic |
| KT339908 | SAMN11519417 | NC | Mid-Atlantic |
| KT339909 | SAMN11519418 | NC | Mid-Atlantic |
| KT339910 | SAMN11519419 | NC | Mid-Atlantic |
| KT339911 | SAMN11519420 | NC | Mid-Atlantic |
| KT339912 | SAMN11519421 | NC | Mid-Atlantic |
| KT339913 | SAMN11519422 | NC | Mid-Atlantic |
| KT339914 | SAMN11519423 | NC | Mid-Atlantic |
| KT339915 | N/A | NC | Mid-Atlantic |
| KT339916 | SAMN11519425 | NC | Mid-Atlantic |
| KT339917 | SAMN11519426 | NC | Mid-Atlantic |
| KT339918 | SAMN11519427 | NC | Mid-Atlantic |
| KT339919 | SAMN11519429 | NC | Mid-Atlantic |
| KT339920 | SAMN11519430 | NC | Mid-Atlantic |
| KT339921 | SAMN11519377 | IL | Midwest |
| KT339922 | SAMN11519440 | IL | Midwest |
| KT339923 | SAMN11519378 | IL | Midwest |
| KT339924 | SAMN11519383 | IL | Midwest |
| KT339925 | SAMN11519384 | IL | Midwest |
| KT339926 | SAMN11519387 | IL | Midwest |
| KT339927 | SAMN11519388 | IL | Midwest |
| KT339928 | SAMN11519390 | IL | Midwest |
| KT339929 | SAMN11519391 | IL | Midwest |
| KT339930 | N/A | IL | Midwest |
| KT339931 | SAMN11519379 | MI | Midwest |
| KT339932 | SAMN11519393 | MI | Midwest |
| KT339933 | SAMN11519375 | MN | Midwest |
| KT339934 | SAMN11519382 | WI | Midwest |
| KT339935 | SAMN11519376 | IA | Midwest |
| KT339936 | SAMN11519372 | ID | Western |
| KT339937 | SAMN11519437 | ID | Western |
| KT339938 | SAMN11519435 | CO | Western |
| KT339939 | SAMN11519436 | CO | Western |
| KT339940 | SAMN11519438 | CO | Western |
| KT339941 | SAMN11519433 | WA | Western |
| KT339942 | SAMN11519434 | OR | Western |
| KT339943 | SAMN11519371 | OR | Western |
| KT339944 | SAMN11519439 | OR | Western |
| KT339945 | SAMN11519373 | CA | Western |
| KT339946 | SAMN11519374 | CA | Western |
| KT339947 | SAMN11519411 | ME | Northeast |
| N/A | SAMN11519355 | NC | Mid-Atlantic |
| N/A | SAMN11519356 | NC | Mid-Atlantic |
| N/A | SAMN11519357 | NC | Mid-Atlantic |
| N/A | SAMN11519358 | NC | Mid-Atlantic |
| N/A | SAMN11519359 | NC | Mid-Atlantic |
| N/A | SAMN11519360 | NC | Mid-Atlantic |
| N/A | SAMN11519361 | NC | Mid-Atlantic |
| N/A | SAMN11519362 | NC | Mid-Atlantic |
| N/A | SAMN11519363 | NC | Mid-Atlantic |
| N/A | SAMN11519364 | NC | Mid-Atlantic |
| N/A | SAMN11519365 | NC | Mid-Atlantic |
| N/A | SAMN11519366 | NC | Mid-Atlantic |
| N/A | SAMN11519367 | NC | Mid-Atlantic |
| N/A | SAMN11519368 | NC | Mid-Atlantic |
| N/A | SAMN11519369 | NC | Mid-Atlantic |
| N/A | SAMN11519370 | NC | Mid-Atlantic |
| N/A | SAMN11519380 | OH | Midwest |
| N/A | SAMN11519428 | NC | Mid-Atlantic |
| N/A | SAMN11519444 | VT | Northeast |
| N/A | SAMN11519445 | NH | Northeast |
| N/A | SAMN11519447 | NH | Northeast |
| N/A | SAMN11519448 | MD | Northeast |
| N/A | SAMN11519449 | VT | Northeast |