Protocol:

EXPERIMENTAL MEASUREMENT OF DUNG REMOVAL RATE ON A WORLDWIDE SCALE

1. INTRODUCTION

Dung beetles are key components for the functioning of many ecosystems, as they are involved in several ecosystem services due to their roll in dung removal. Dung removal is a very important process for the functioning of ecosystems as it improves the nutrient cycling, bioturbation, seed dispersal, plant growth, and parasite regulation. It is usually assumed that functional diversity (i.e. the diversity of the organismal traits that influence ecosystem functioning) and certain traits are directly linked to ecosystem functioning. In this multi-site experiment we want to study the relationship between dung removal rates and the functional composition of dung beetle assemblages in different grasslands around the world. We expect that the composition of dung beetle assemblages, and in particular the composition in terms of functional groups based on dung processing behavior (dwellers, tunnellers and rollers vs. big, medium and small sizes), will be related to dung removal efficiency.

role

1extra: "control of dung-breeding organisms" (me refiero a regulación de las poblac. de moscas (ejem. australiano)

la coma va antes y tras "in particular"

marco con circulito la puntuación q sobra

en este caso, esta coma separa SUJETO y VERBO! "We expect that A and B will be related to C", así q sobra.

Land use changes are one of the main agents of global change. The progressive intensification of human-related activities can negatively affect the diversity of local communities and of the ecosystem services they provide. In this project we will also consider different levels of land management, evaluating the effects of land use intensification on the diversity of dung beetle assemblages and on the rates of dung removal. We expect that grasslands with lower management intensity (less amount of cattle per area) will have a more complex assemblage structure, higher diversity and dung removal rates.

The added value of working at this large scale is the inclusion of a wide range of climates, evolutionary histories and human impacts. This will also allow evaluating whether latitudinal changes will have an impact on this process and on dung beetle assemblage composition. We expect that dung beetle functional diversity, abundance, richness and biomass will show the typical latitudinal gradient, with higher diversity towards the tropics; such gradient should affect dung removal rates. Confounding factors like herbivores and habitat type, and dung pad size will be controlled for as much as possible, by using size- and volume-controlled dung pads of one herbivore species in a grassland environment.

document

In summary, in this project, we propose a sampling protocol that will be used in a worldwide experiment with multiple sites in different biogeographical and climatic regions. Such protocol will allow evaluating the following research questions: (i) Is there a relationship between dung removal and dung beetle assemblage structure? (ii) Does cattle management intensity affect dung removal? (iii) Are these patterns similar or different around the world?

answering/addressing

(iii) no me queda claro, te refieres a "patterns of cattle intensity?" o a algo así como: "Is there a global variation in the link between assemb-struct <~> dung-remov-efficiecy?"

2. FIELD EXPERIMENTAL DESIGN

2.1. Habitat (one locality with two different conditions)

todo en plural? habitatS, grasslandS?

- The main habitats for this study are the grasslands that are used for cattle production.
- Two sampling points: one grassland with intensive use (mainly grassland – open habitat) that has a higher amount of cattle per area (≥ 4 heads per hectare/100 m^2) vs. one grassland with extensive use (grassland with shrubs – close habitat) that has a lower amount of cows per area (≤ 2 heads per hectare/100 m^2) and in some regions might even have natural vegetation.

no estoy muy seguro de q en inglés se use "heads"

hectare o 100m² son alternativas? una hectarea son 10.000m²

- The minimal experimental unit is one locality with two different conditions of grasslands. If any research group wants to do more than one locality with another two grasslands or want to include a third grassland in the same locality they are free to do it.

wantS

extra: incluir un área mínima de grassland. hay "potreros" MUY pequeños

2.2. Experimental design

2.2.1 Dung

The dung that you will use in the experiment will act as an attractant to dung beetles, making dung quality a decisive factor in the success of the experiment. Dung beetles are attracted to fresh dung, so make sure that the dung that you use is fresh (collected very recently; ideally it should have been just defecated to avoid having any dung beetle on it) and not dry. In addition, several literature sources report that the presence of anthelmintic (e.g. Ivermectin) may have a positive or a negative effect on dung beetles attraction to dung, so you must try to use anthelmintic free dung. Each experimental unit, control and pitfall will have 500 gr of fresh cow dung, without beetles (if you could not be able to find fresh cow dung you could freeze it at 0 C° for at least 48 hours, to ascertain that the dung contains no living dung beetle).

beetleS

anthelmintic-free

are

freeze it in advance at...

2.2.2 Dung removal rates: experimental and control units

¿es necesaria esta aclaración de unidades?

The experimental units consist of a bucket of 10 L (10000 ml) burrowed in the soil and filled with soil taken from the same place. 500 g of fresh cow dung will be placed over the surface of the soil in the center of the bucket (Figs. 1a,b and Annexe 1). When weighting the cow dung it is important to have a digital pocket scale that works with batteries and that measures two decimal positions (0.01g). These experimental units will be left in the field for 72 hours. After this period, weight the remain dung left on the surface of the bucket. Then, carefully remove the soil from inside of each bucket and place it on a white piece of fabric on the ground, analyzing it carefully and collecting (with a lot of care and patience) all the dung fragments and pieces that you will find. After collecting all the possible fragments weight them. Release the dung beetles that you might find inside the bucket.

weighing
el sustantivo "peso" si tiene T "weighT"

el verbo "pesar" no lleva T "weigh"

weigh the remaining dung

duda:
deben pesar los fragmentos juntos o cada uno por separado??

On each sampling point you will also set control units that will allow evaluating how much weight would be lost by evaporation. Each control unit consist in 500 g of fresh cow dung placed on top of the ground, but it must be totally covered with a very fine polyester white or green mesh fabric (mosquito net – diameter of holes should be less than 0.2 cm) so it would be impossible for any dung beetle to get inside (Fig. 1c

consistS of

the ground surface

creo q despista mejor 2mm

to avoid any bettle getting inside

compadre Noriega, estoy intentando corregir las cosas más graves del inglés.
pero me dijiste q ya se lo vas a enviar a alguien para la parte de idioma, no?
si es así, me puedo centrar en la parte científica, sino, ya me dirás y en otro momento me centro en idioma.

and Annexe 2). It is important to dig in the borders and buried the net at least 5 to 10 cm in the ground to prevent dung beetles diggers to enter the enclosure. These control units will also be left in the field for 72 hours, simultaneously with the experimental units. After this period weight the dung still left at the surface.

bury
tunnellers (paracópridos, no?)

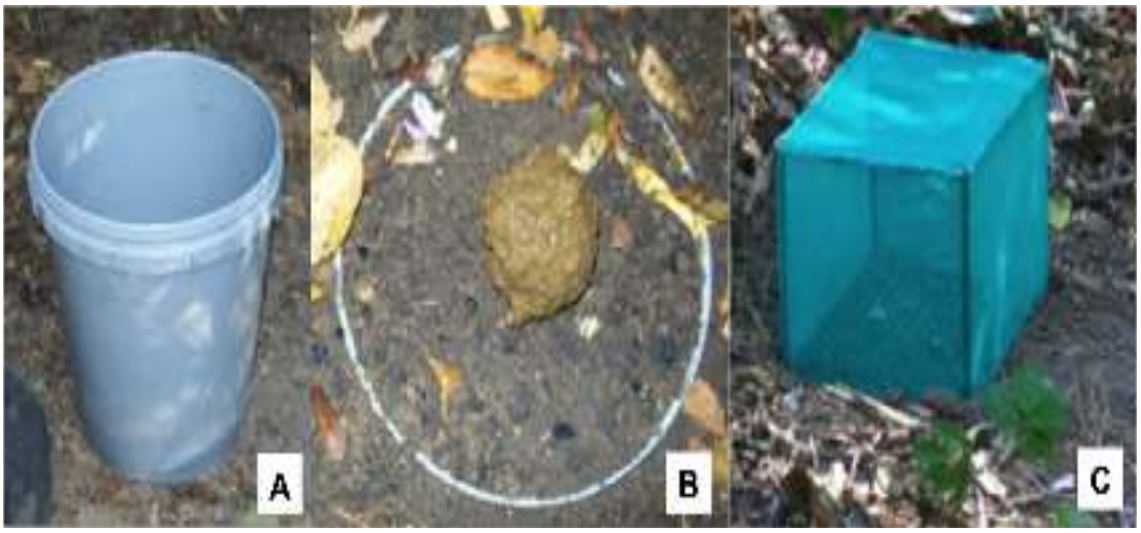

Figure 1. Experimental unit (A and B) and control unit (C) used to measure dung removal rates.

frase poco clara, un poco de lio, propongo:
experimental unit (a,b) to measure dung removal rate
control unit (c) to measure evaporation rate.

2.2.3 Experimental setting

On each sampling point (grassland condition) set a 450 m transect, composed by 10 experimental units that will be placed at 50 m from each other (Fig. 2). Three control units will be set 50 m apart from this transect (Fig. 2). Both experimental and control units will be left in the field for 72 hours.

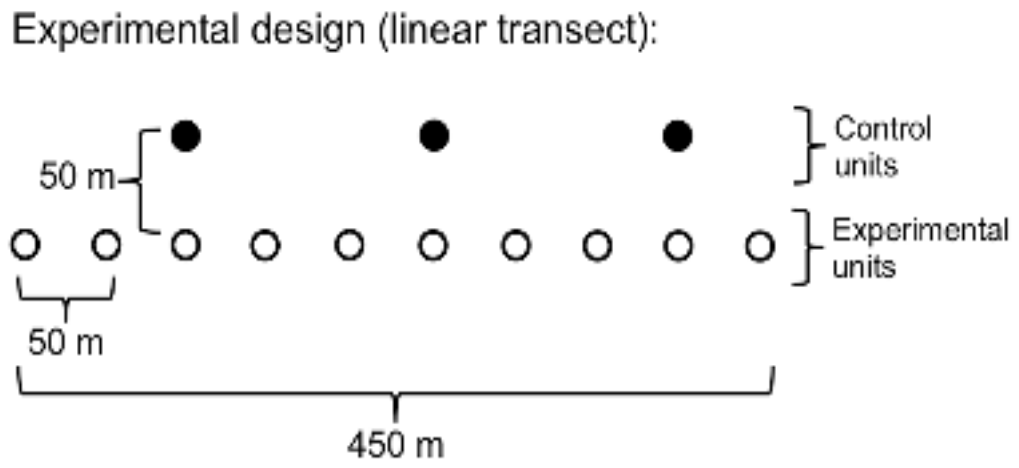

Figure 2. Setting of the experimental linear transect with experimental and control units.

2.2.4 Sampling of dung beetle assemblages

Dung beetle assemblages can be sampled right after performing the dung removal experiments. In fact, this part of the protocol can be set the same day the experiment described above is removed from the field, the next day or 24 hours after. It is important to do the sampling during the next 48 hours after the experiment to keep the same weather conditions. Sampling will consist of a transect with 10 pitfall bait traps (Fig. 2 and Annexe 3). Each trap will be a plastic pot of 1L (1000 ml) that will be buried at ground level and filled with approx. 500 ml of water (depending on weather conditions) + approx. 2 spoons of kitchen salt (NaCl) + approx. 1/2 spoon of scentless soap (preservation fluid). You can re-use the holes that were done for the dung removal experiment. On top of this, you will place a squared plastic or metallic chicken mesh of 15 x 15 cm, that will be pinned down to the ground by four metal wires set at each corner of the mesh. Finally, place 500 g of fresh cow dung on top of this mesh (Fig. 3). Collect the samples 72 hours later, placing each sample in a individual container, and marking each sample with a pencil in a piece of paper. Finally remove the traps.

te refieres a meteorología?
o a estación del año?
o en ambiente en general?

10 baited pitfall traps

hace falta la aclaración de unidades?

creo q es más fácil
indicar las cantidades
para 5 o 10 L de agua
y así en el campo solo
hay q echar el agua
ya preparada en las
trampas

qué hay q marcar?
el nombre de la trampa en un papel y echarlo al bote?
apuntar observaciones en un cuaderno de campo?
apuntar...?

me parece casi imposible colocar
500g de excremento de vaca
en una malla de 15x15cm
y además q sobre malla sin excrm.!!

la experiencia q tengo es q 200-300g
ocupan un diametro de 13x13cm aprx
(sin dejar malla libree!!!)

para conseguir dejar algo de malla-libre
deberías aumentar la malla a 25x25cm
y así q te quepan 500g en el centro

eso te obliga a usar un vaso mucho mayor!
al menos de diámetro, y supongo q volumen!

Figure 3. Pitfall trap design.

2.2.5 Sampling procedures (summary)

1. Choose two grasslands with different management intensities: intensive vs. extensive management.
2. The sampling design of the position of the experimental units will depend on the area in which each team will work. We recommend a linear transect of 10 experimental units. The distance between each experimental unit must be of 50 m, so the linear transect will be of 450 m (Fig. 2).
3. For each experimental unit dig a hole and with that soil fill the bucket (10 L).
4. Weigh 500 gr of fresh cow dung for each experimental and control unit and place it on top of it.
5. Set three control units 50 m apart from the experimental transect (see Fig. 2), following the point 4 described above.
6. Cover the control units with a mesh that will protect the dung from any insect.
7. After 72 hours, weight the dung of the experimental and control units.

8. Remove experimental and control units.
9. Carefully remove the soil from the experimental units, place it on top of a piece of white fabric, collect (with a lot of care and patience) all the fragments and pieces of dung that you can find, and weight them in the balance.
10. In the same place where the experimental units were set, install the pitfall traps with 500 gr of fresh cow dung.
11. After 72 hours collect each sample from each trap in a single container (plastic bag or jar) with ethanol (70-96%), mark each sample with a pencil in a piece of paper and remove the traps.

The total amount of time in the field will be around 8 days of work, and two or three persons can easily do all the fieldwork. You can use the following timetable:

Day 1	Set the dung removal experiment at two sites.
Day 2	(24 hours)
Day 3	(48 hours)
Day 4	Collect data from the dung removal experiments at two sites, assessing first the sites that were set first.
Day 5	Set pitfalls traps on these two sites.
Day 6	(24 hours)
Day 7	(48 hours)
Day 8	Collect the pitfall traps at the two sites.

3. LABORATORY WORK

3.1 Dung beetles identification, measurement of traits, and functional groups

Identify all the dung beetles of each sample to the species level, or to the lowest taxonomic level possible. In case you assign individuals to morph species please try to photograph them. Assign each species to a functional group: Paracoprids (tunnelers), Telecoprids (rollers) and Endocoprids (dwellers) versus the sizes: small (<10 mm), medium (≥10-15 mm) and big (<15 mm). For each species select randomly 10 individuals (If the species that you collect are present in both areas, select 5 and 5 individuals for each grassland condition that you sampled, if not, select 10 individuals for the grassland where that species is present) and measure with a digital caliper nine morphological traits (Fig. 4 and Annex 4): i) **HL**= Head Length; ii) **HW**= Head Width; iii) **PL**= Pronotum Length; iv) **PW**= Pronotum Width; v) **PH**= Pronotum Height; vi) **EL**= Elytron Length; vii) **pTL**= proTibia Length; viii) **pTW**= proTibia Width and ix) **mTL**= metaTibia Length.

AND si quieres reducir su trabajo, esto es fácilmente deducible medidas (HL+PL+

y si no hay suficientes te importa q cojan 3 y 7 individuos? 2+8?

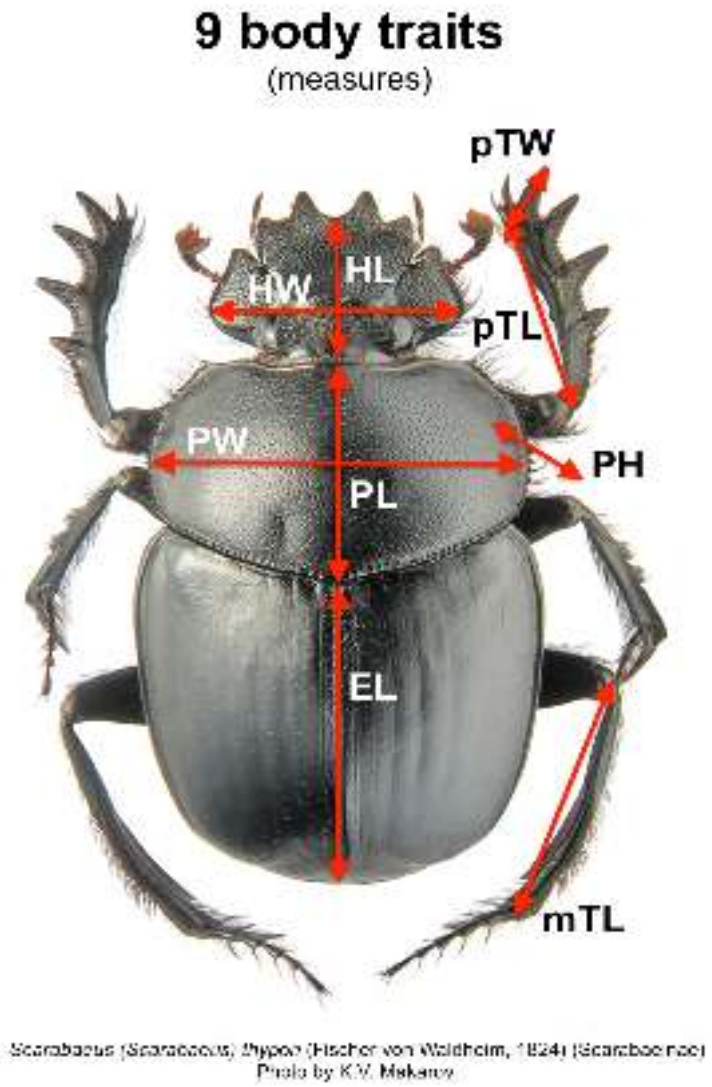

Figure 4. Description of the traits that need to be measured

3.2 Dung beetles biomass

extra: no les vas a pedir q sean muy cuidadosos de limpiar la tierra adherida de cada individuo??

Use the same 10 individuals randomly chosen before to measure biomass. Dry them at 80º for 72 hours. If the species is present in both grassland conditions, weight in a precision digital scale 5 individuals from each grassland condition simultaneously and divide the total weight by 5, if not, measure 10 individuals simultaneously for each species present in just one grassland condition.

este es el "weight" q SI lleva T

4. INFORMATION THAT YOU SHOULD SEND US

The deadline to participate in this worldwide experiment is December 2016, so you will have one year to do the experiment and the sampling in one locality with two grassland conditions. We need to know the history of use of the area (last 10-20 years) that you selected for the experiment. Please answer these questions with much detail and accuracy as possible:

landuse

as

por eso falta GPS en checklist

- Precise geographical coordinates?
- Which is approx. the cattle density per site (number of cows per area)? hectare or 100m²?

si mi veterinaria no me falla... todas las vacas son la misma sp. pero diferente raza ("breed")

- Which species/type of cattle uses that site?
- Did the ranchers use any kind of anthelmintic (Ivermectin) with the cattle? What type? Frequency, amount and how long have they used it?
- How is the weather? Seasonality? Precipitation regime? climate
- What is the average temperature?

PRECIPITATION ??

- What is the average humidity percentage?
- How long has the site been used for pasturing?
- Which type of ecosystem can be found near or in the surroundings of the grassland?
- Is there any kind of vegetation in the grassland? Type of vegetation? Which is the dominant species? What is the approx. percentage between arboreal vs. herbaceous?
- Which is the type of soil?

Regarding the experiments we will need to know:

creo q sería una idea buenisima q les enviases una tabla excel como plantilla para q rellenen los resultados NO ?? además de ayudarles a ellos tendrías un formato estádar de todos los participantes.

- Total weight of the dung of each experimental unit after the experiment is finished. Please also refer separately to the weight from the dung that was left on top of the unit and the weight of the dung found in the soil that was inside the bucket.
- Total weight of the dung of each control unit after the experiment is finished. creo q este "total" despista, si quieres el peso de CADA unit, déjalo sin "total"
- The species identification of each individual found on each pitfall trap.
- The abundance of each species found on each pitfall trap.
- Assignation of each species to functional group: Paracoprids (tunnelers), Telecoprids (rollers) and Endocoprid (dwellers) versus sizes (big, medium and small).
- The measurement of the nine traits described above for 10 individuals (5 and 5 individuals from each grassland condition) for each species.
- The biomass (g, with three decimals) for each species founded (5 and 5 of each group of 5 individuals from each grassland condition).

Finally, if you have any doubt or any question related with this protocol please contact us as soon as possible. We will be more than happy to help you and try to solve any possible problem.

Contact information:

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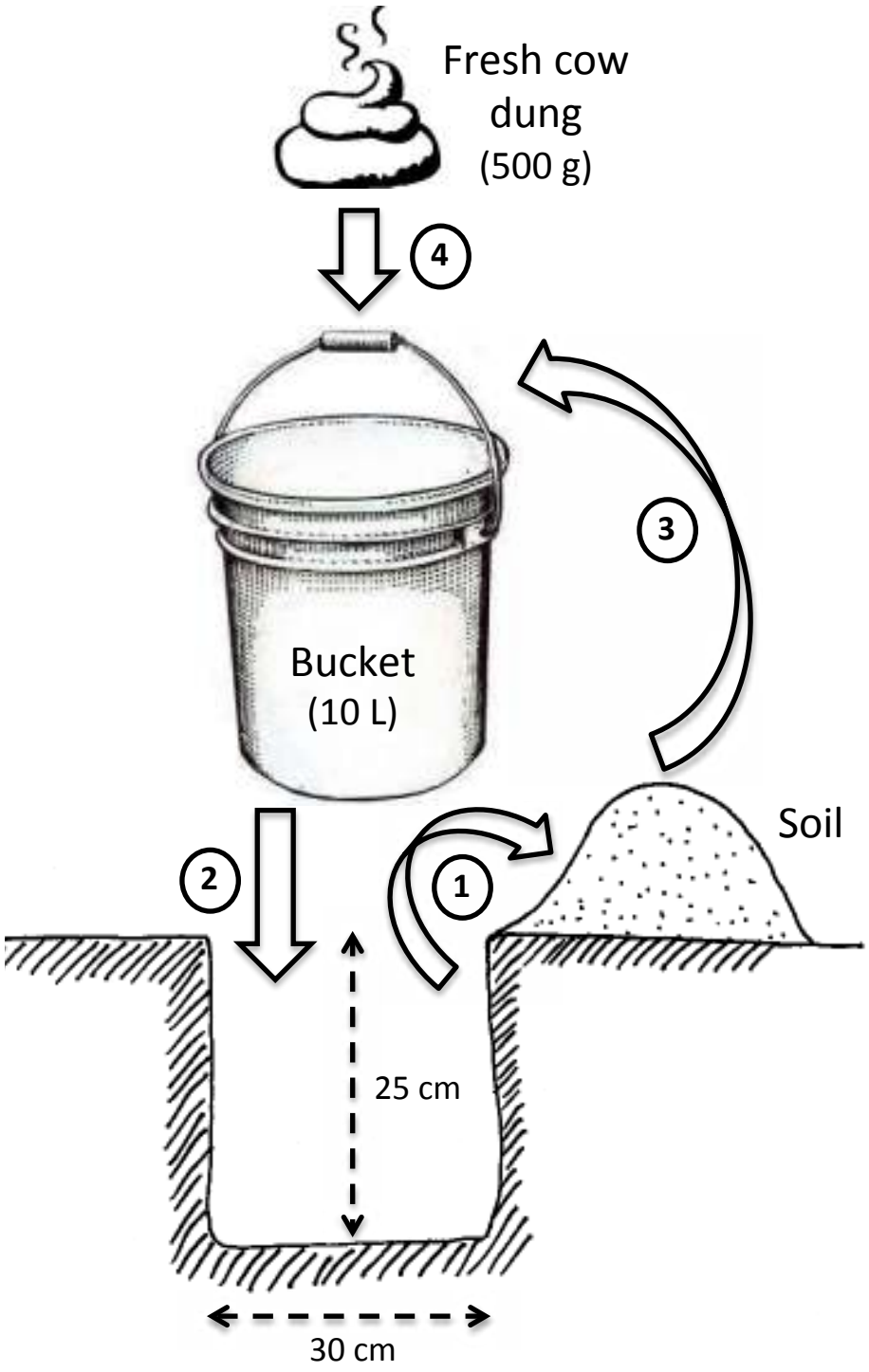
Annex 1: Experimental Unit



Example:

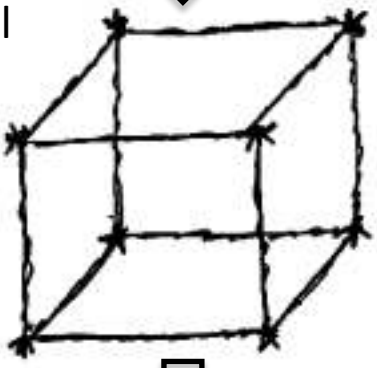
1. For each experimental unit dig a hole.
2. Put the bucket in the hole.
3. Fill the bucket (10 L) with the soil of the hole.
4. Weigh 500 gr of fresh cow dung and place it on top of it.

Annex 2: Control Unit

Fine polyester green mesh fabric

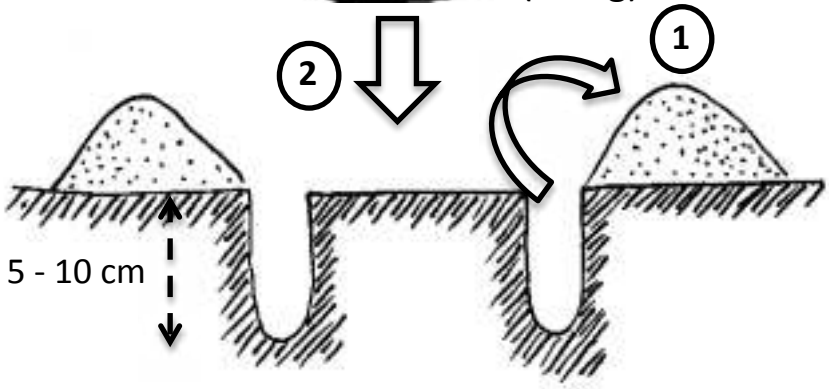
Example:

Wire or steel bars cubic structure



or

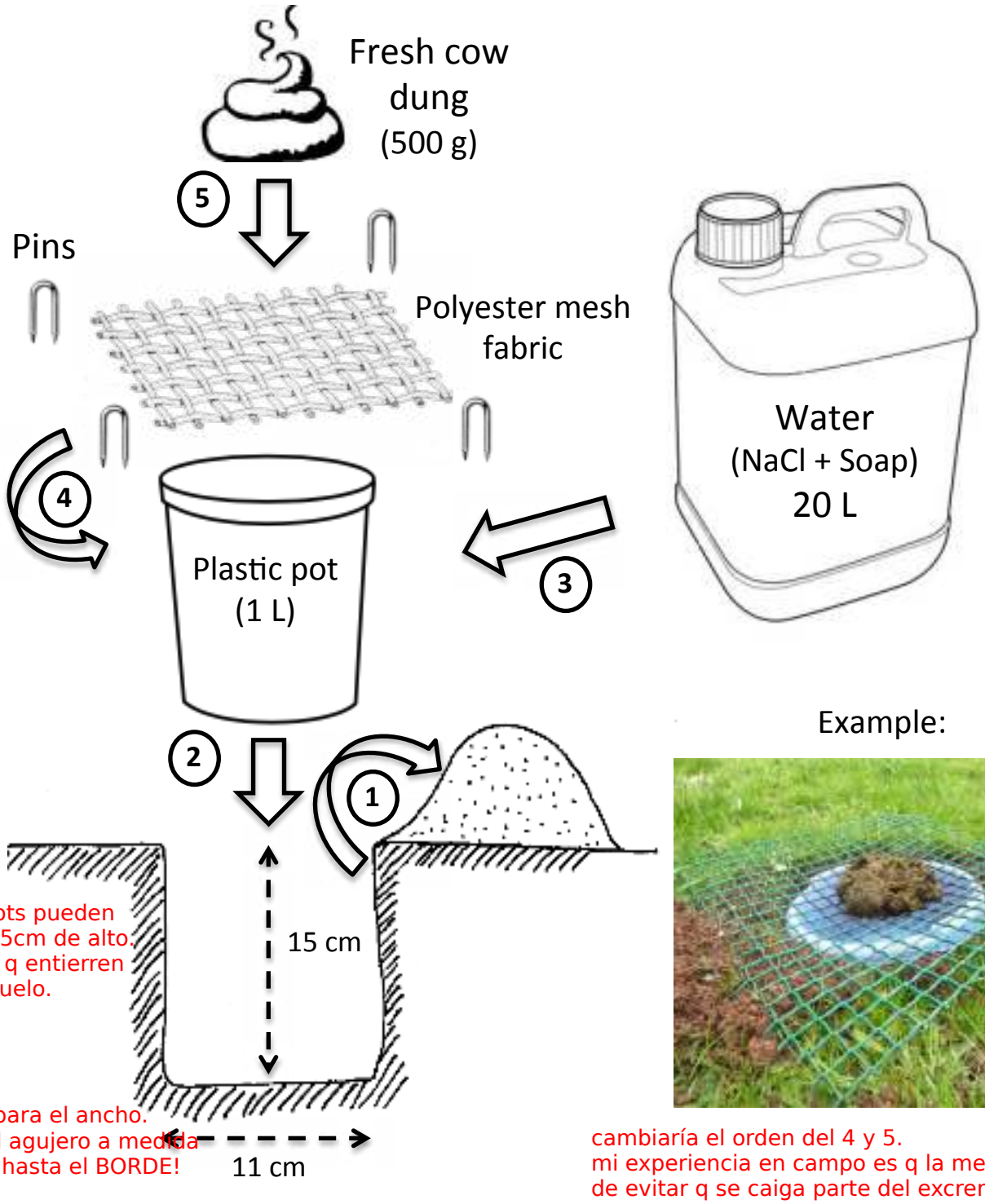
Fresh cow dung (500 g)



1. For each control unit dig a furrow around a small platform.
2. Weigh 500 gr of fresh cow dung and place it on the soil.
3. Put the wire cubic structure over the cow dung.
4. Put the polyester mesh fabric over the cubic structure covering it and buried the borders of the fabric in the furrow and cover it with soil.

1. esto es dificil de explicar. quizá diciendo "circular furrow" o con una foto/diagrama en prespectiva 3D?
3-4. ¿cómo propones FIJAR la mosquitera sobre la estructura? con bridas de plástico? otra manera?
no crees q es dificil FIJAR la mosquitera sobre la estructura en el campo? quizá mejor hacerlo en el labo y llevar las estructuras con la mosquitera ya montada?
en ese caso: 3. place the structure over the dung and IN the furrow
4. add soil to bury the borders of the fabric in the furrow

Annex 3: Pitfall bait trap



Example:

algunos pots pueden no tener 15cm de alto. mejor pon q entierren al ras de suelo.

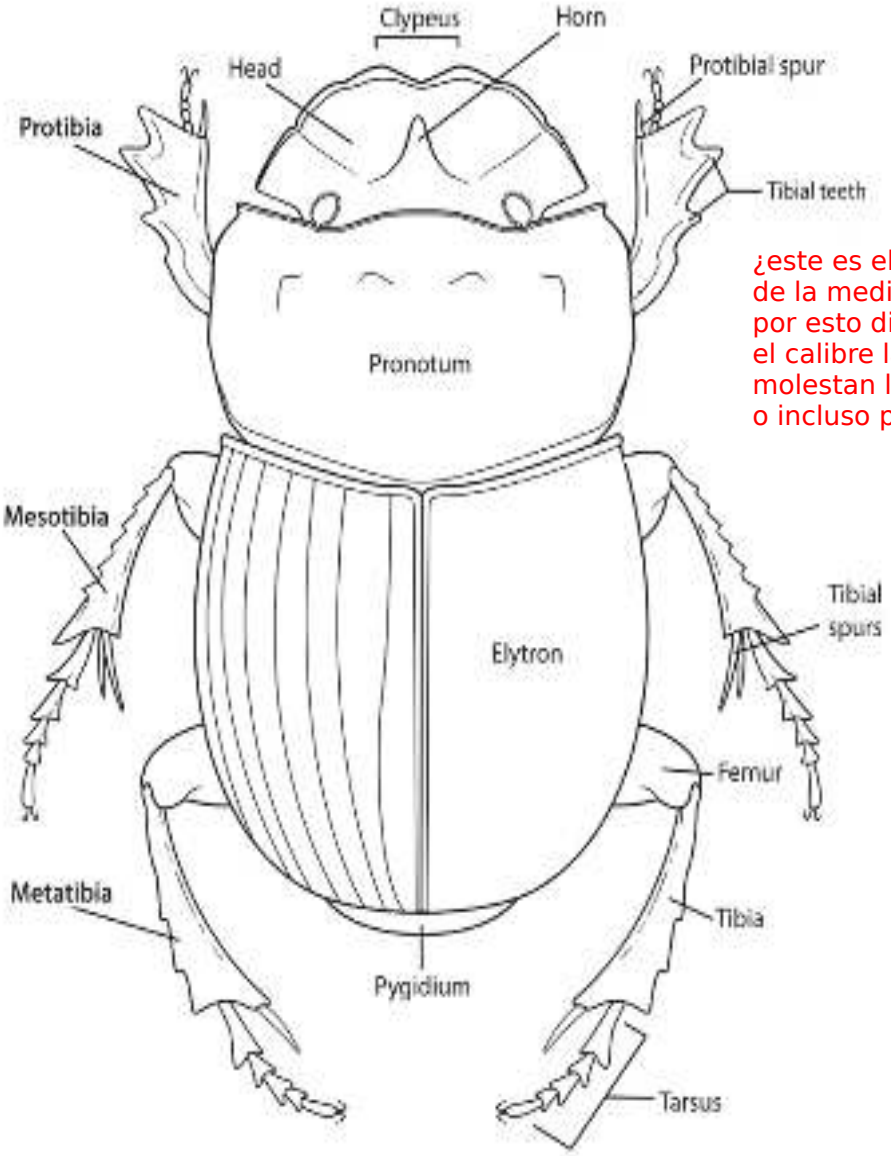
lo mismo para el ancho.
q hagan el agujero a medida y rellenen hasta el BORDE!

cambiaría el orden del 4 y 5.
mi experiencia en campo es q la mejor manera de evitar q se caiga parte del excremento dentro es poner el excremento sobre la malla y LUEGO colocar la malla sobre la trampa.
(sobre todo con excremento de vaca "fresco-fluido"

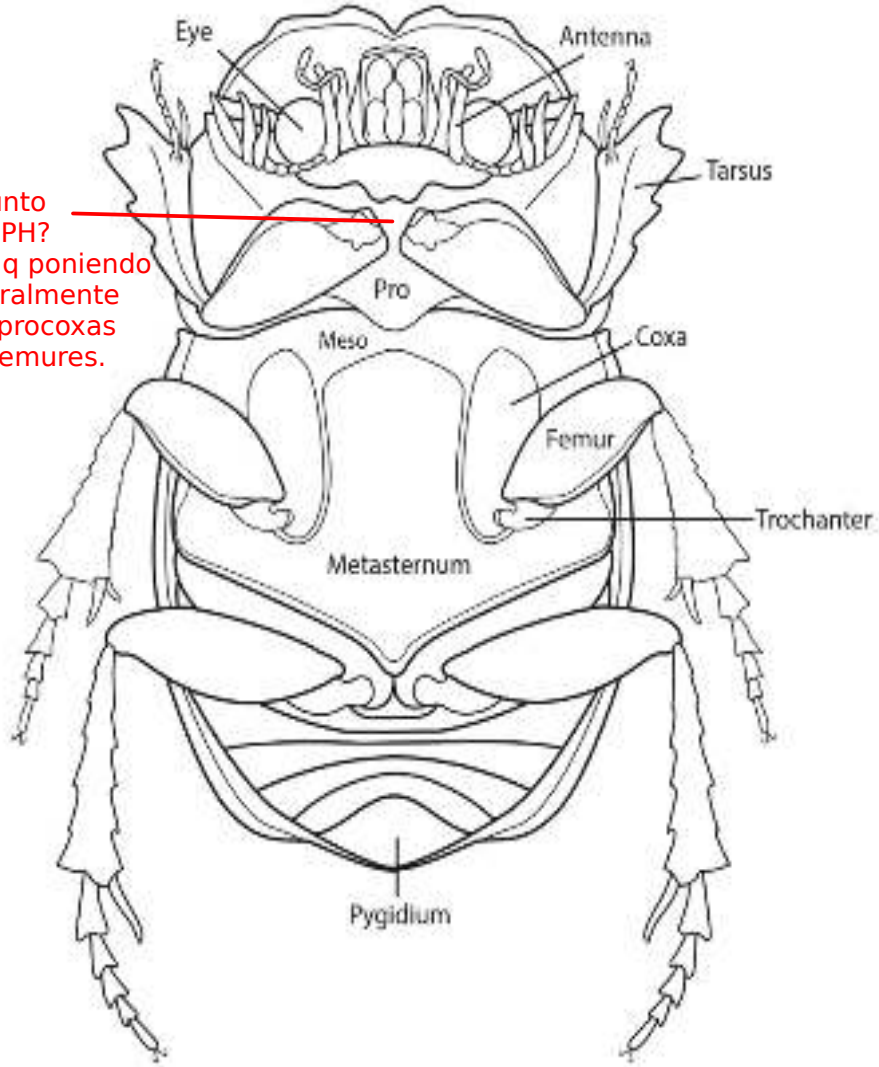
1. For each pitfall trap dig a hole.
2. Put the plastic pot (1 L) in the hole.
3. Fill the pot with 500 ml of water (NaCl + soap).
4. Put the polyester mesh fabric with the pins over the plastic pot.
5. Weigh 500 gr of fresh cow dung and place it on top of the polyester fabric.

Annex 4: General anatomy & Traits

(main body parts)



¿este es el punto de la medida PH? por esto digo q poniendo el calibre lateralmente molestan las procoxas o incluso profemures.



HL & HW

(measures)

HW:

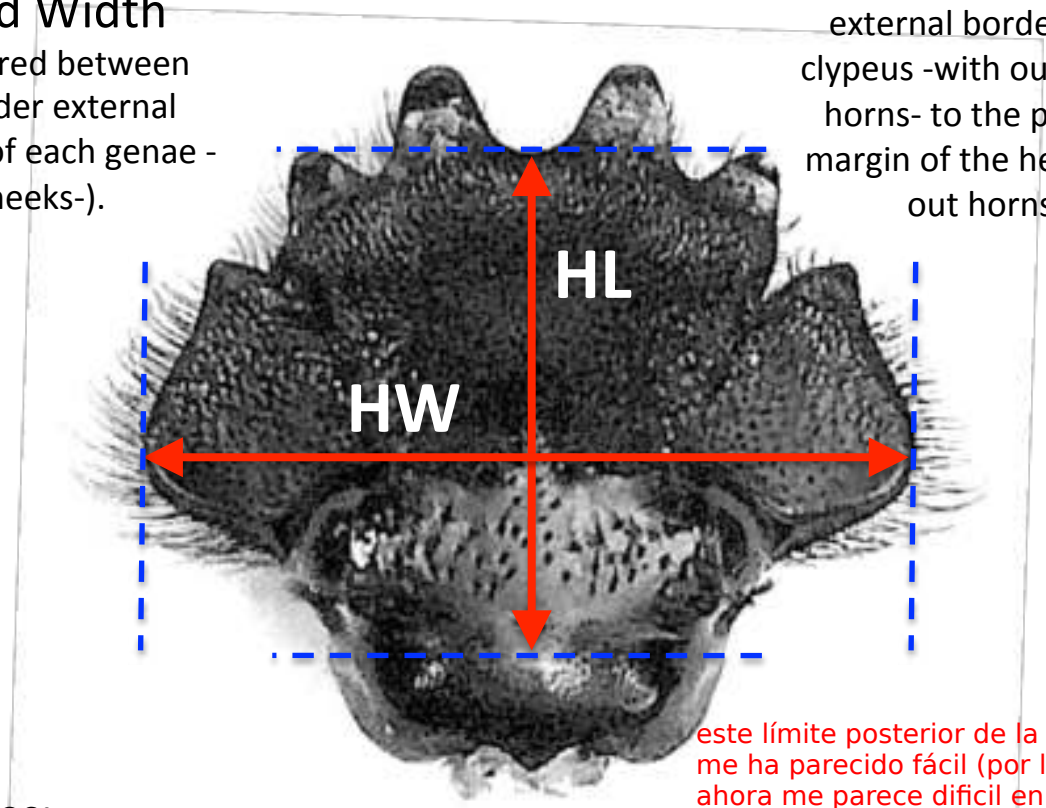
Head Width

(measured between the wider external borders of each genae -cheeks-).

HL:

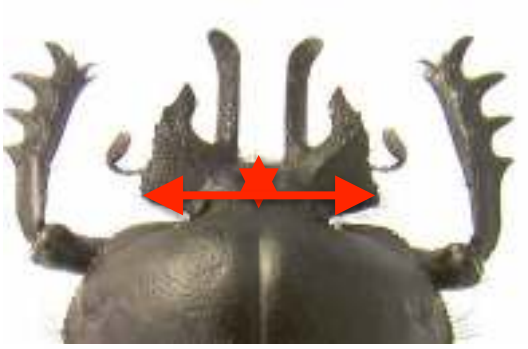
Head Length

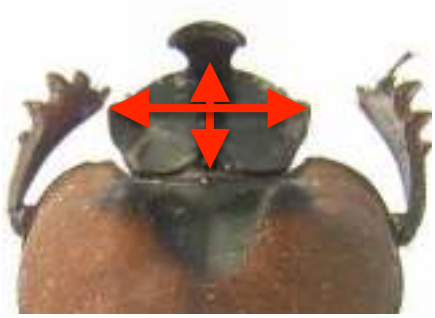
(measured from the external border of the clypeus -with out teeth or horns- to the posterior margin of the head –with out horns-).



este límite posterior de la cabeza q siempre me ha parecido fácil (por la carena de Ontho) ahora me parece dificil en algunos géneros. pero no tengo ninguna propuesta.

Examples:

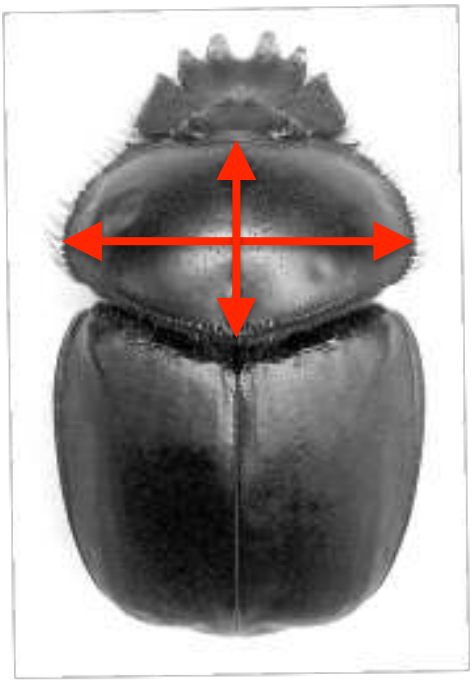




Example photos taken from: Vaz-de-Mello, F.Z., W.D. Edmonds, F.C. Ocampo & P. Schoolmeesters. 2011. A multilingual key to the genera and subgenera of the subfamily Scarabaeinae of the New World (Coleoptera: Scarabaeidae). Zootaxa, 2854: 1-73.

PL & PW

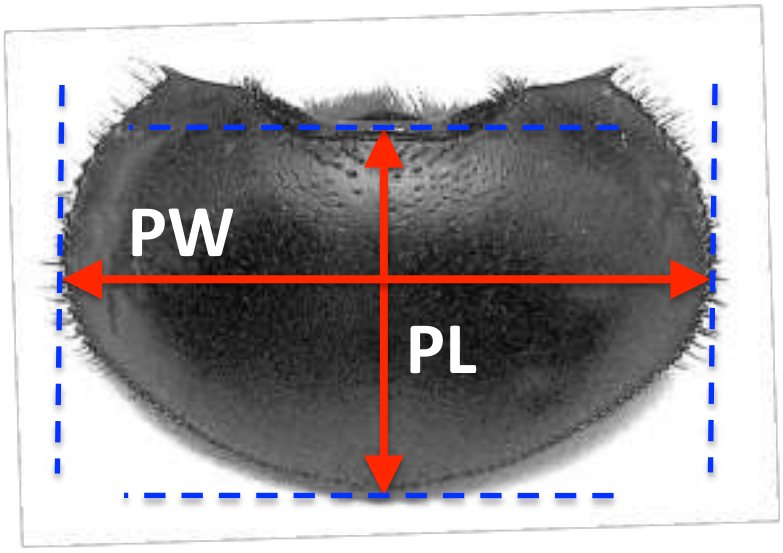
(measures)



PW:

Pronotum Width

(measured between the wider external borders of the pronotum).

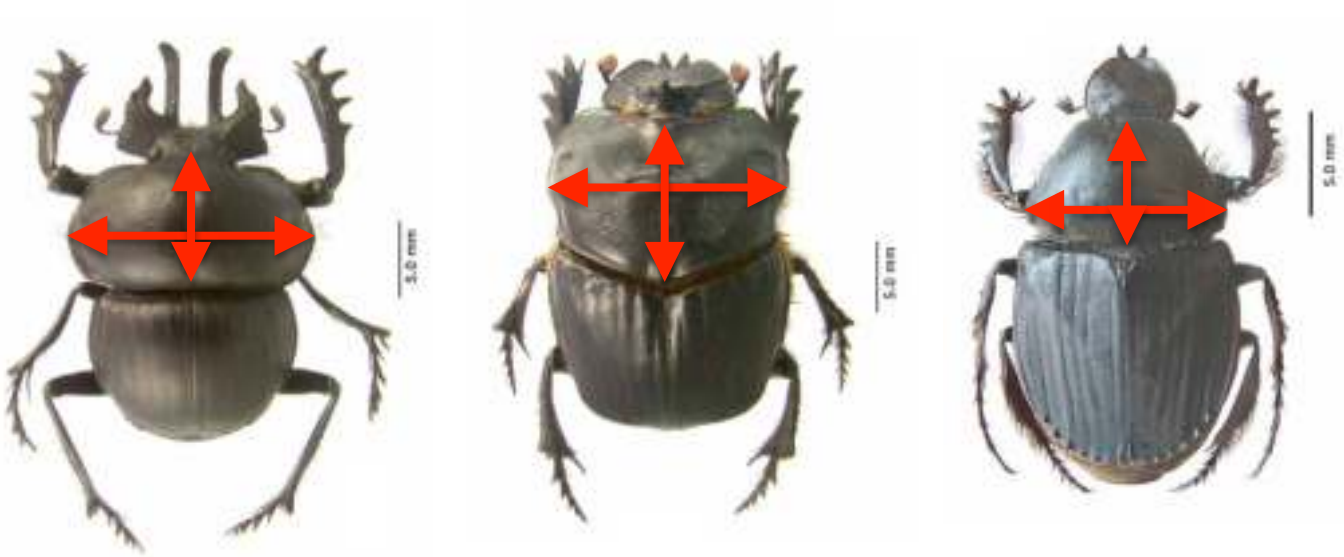


PL:

Pronotum Length

(measured from the anterior border of the pronotum to the posterior border of the pronotum -in the center-).

Examples:



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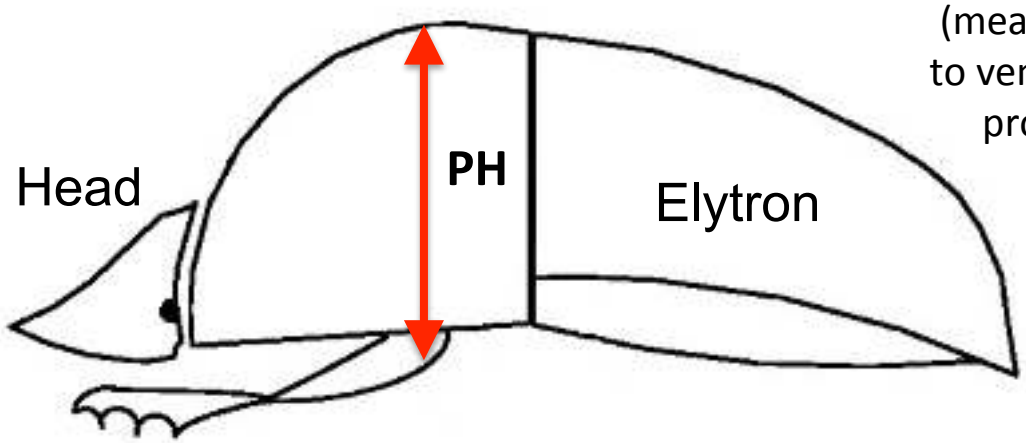
PH

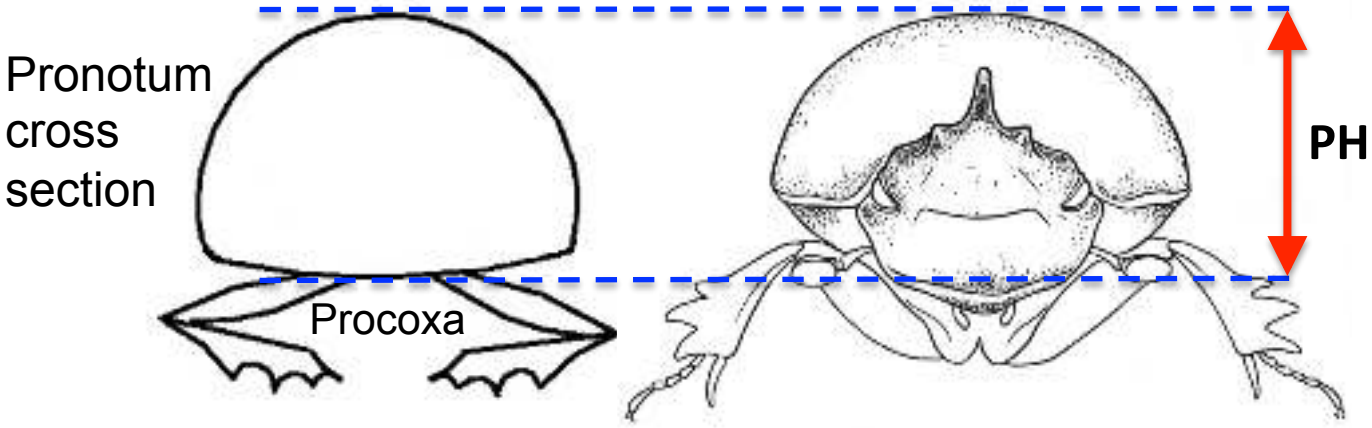
(measures)

PH:

Pronotum Height

(measured from dorsal to ventral surface at the procoxal position)





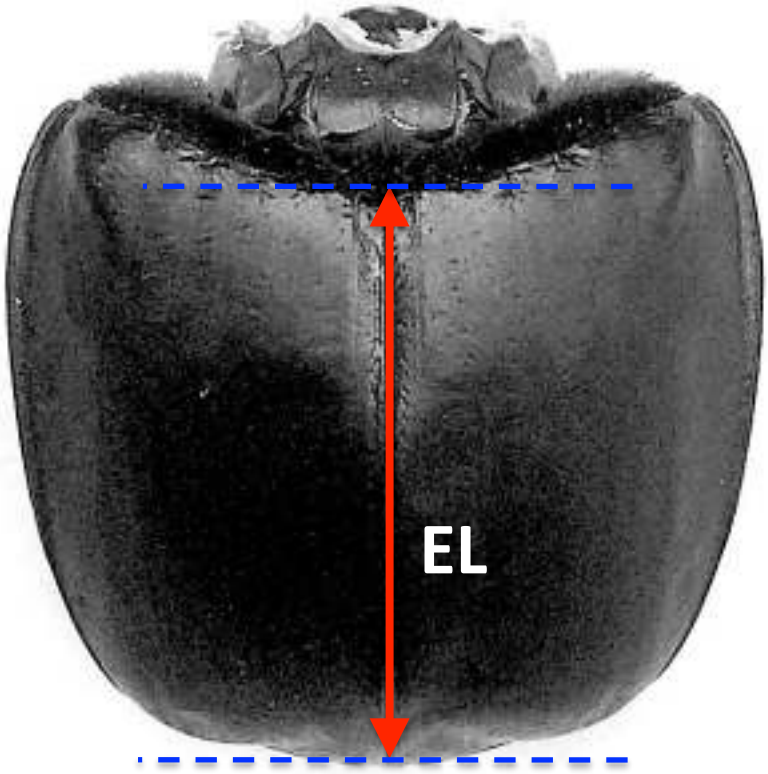
Examples:

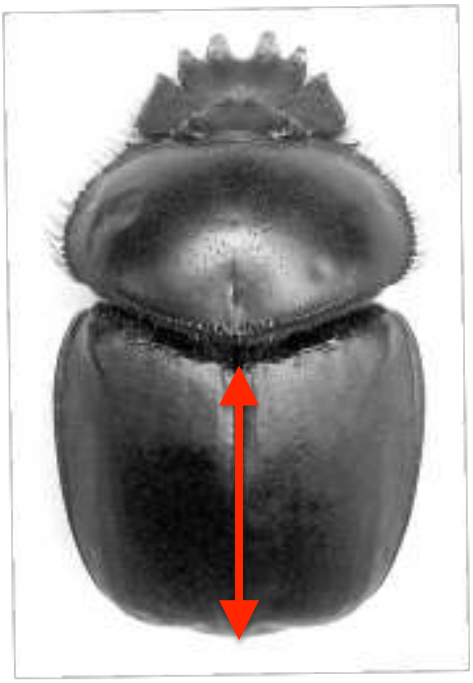
este trait me parece dificil, por estas razones:
-subjetivo en cuanto a la dirección de la flecha. ¿se mide desde el prosternum entre las 2 procoxas hasta...? el máximo? el centro del pronoto? el borde posterior del pronoto?

-suponiendo q se entienda la flecha dibujada... ¿cómo pones el calibre? por delante del bicho? si pones el calibre lateralmente te molestan las patas, las propias procoxas.
Quizá molaría poner una foto del calibre en posición (incluso para el resto de medidas)

EL

(measures)





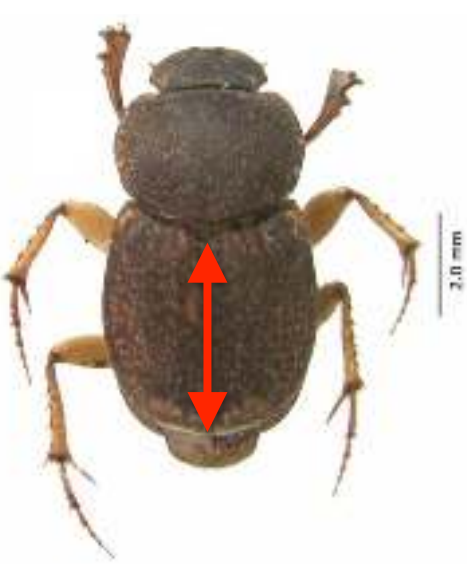
EL:

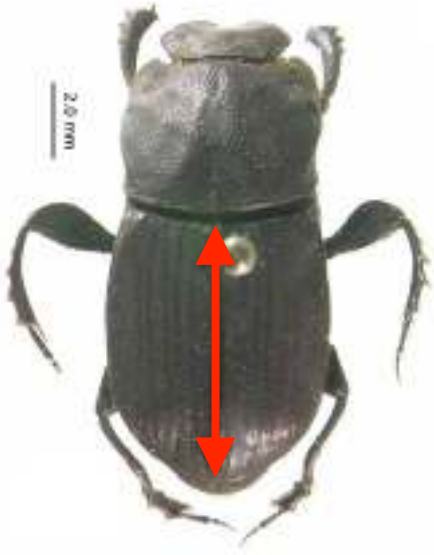
Elytron Length

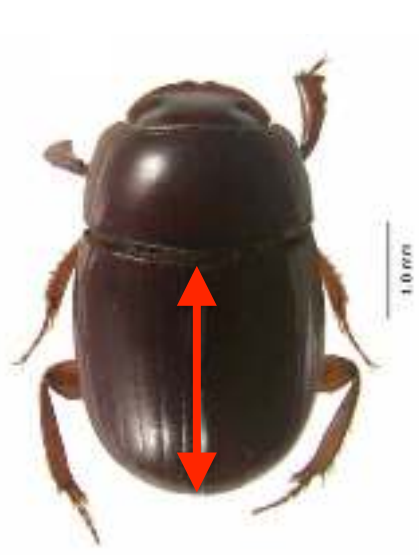
(measured from the anterior border of the elytron to the posterior border of the elytron -in the center without counting the pygidium-).

contando el escutellum o sin contarlo? parece q en el ejemplo de la izquierda omites el escutelo.

Examples:





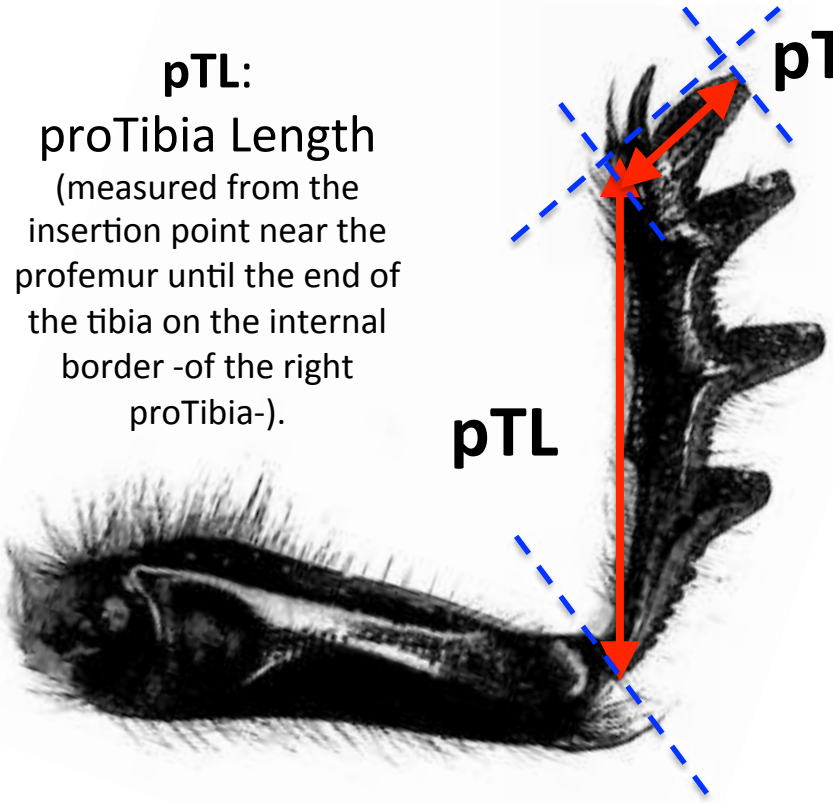


Example photos taken from: Vaz-de-Mello, F.Z., W.D. Edmonds, F.C. Ocampo & P. Schoolmeesters. 2011. A multilingual key to the genera and subgenera of the subfamily Scarabaeinae of the New World (Coleoptera: Scarabaeidae). Zootaxa, 2854: 1-73.

pTL & pTW

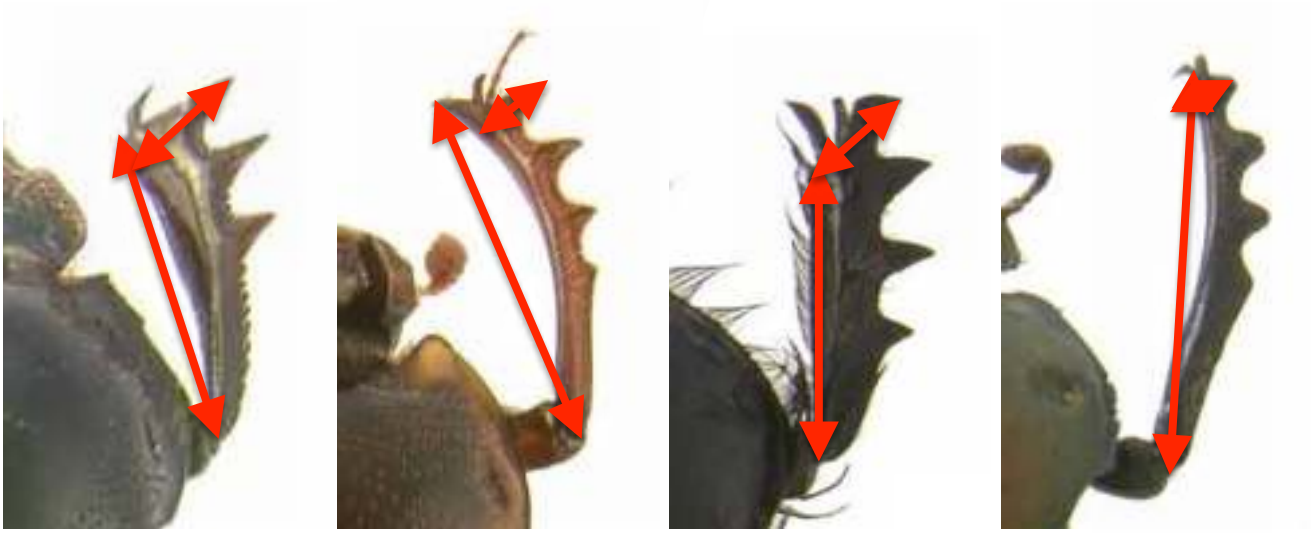
(measures)

pTL:
proTibia Length
(measured from the insertion point near the profemur until the end of the tibia on the internal border -of the right proTibia-).



pTW:
proTibia Width
(measured from the external border of the first tibial tooth to the internal border of the tibia –on the right proTibia-).

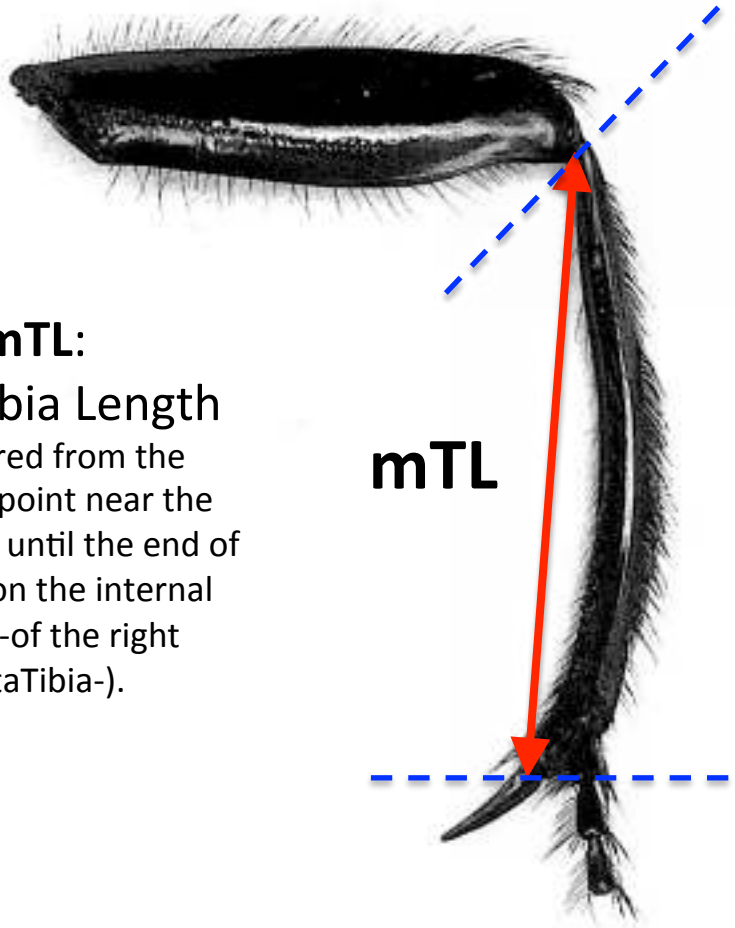
Examples:



Example photos taken from: Vaz-de-Mello, F.Z., W.D. Edmonds, F.C. Ocampo & P. Schoolmeesters. 2011. A multilingual key to the genera and subgenera of the subfamily Scarabaeinae of the New World (Coleoptera: Scarabaeidae). Zootaxa, 2854: 1-73.

mTL

(measures)



mTL:

metaTibia Length

(measured from the insertion point near the metafemur until the end of the tibia on the internal border -of the right metaTibia-).

Examples:

Example photos taken from: Vaz-de-Mello, F.Z., W.D. Edmonds, F.C. Ocampo & P. Schoolmeesters. 2011. A multilingual key to the genera and subgenera of the subfamily Scarabaeinae of the New World (Coleoptera: Scarabaeidae). Zootaxa, 2854: 1-73.

Annex 5: Materials Checklist

Experimental and Traps Material:

- ☐ 20 buckets of 10 L.
- ☐ 1 or 2 shovels to dig holes.
- ☐ Six squares of 1 m^2 of fine polyester white or green mesh fabric (mosquito net – diameter of holes should be less than 0.2 cm) to build a structure that will be used to cover the controls.
- ☐ Large barrel (20 L) to transport the dung.
- ☐ Approx. 23 kg of fresh cow dung (20 experimental units x 500 g + 6 control units x 500 g for dung removal + 20 pitfall traps x 500 g for sampling).
- ☐ Digital pocket scale with a precision of 0.01 g.
- ☐ One hand shovel, tweezers and gloves for handling the dung.
- ☐ 20 plastic pots of 1 L.
- ☐ 20 squared plastic or metal chicken mesh of 15 x 15 cm.
- ☐ 80 iron staple pins; large enough to pin the chicken wire to the ground.
- ☐ 1 k of kitchen salt (NaCl).
- ☐ 1 L of scentless soap.
- ☐ Approx. 20 L of water (depends on weather conditions).
- ☐ 20 plastic bags.
- ☐ Ethanol (70-96%) or other preservative that will allow maintain the dung beetles for long periods.
- ☐ Sieve to separate dung beetles from the water of the pitfall traps.
- ☐ Pencil.
- ☐ Greaseproof paper.
- ☐ Digital pocket scale (precision= 0.01 g).

extra: GPS? por lo menos con el móvil

Laboratory Material:

- ☐ Microscope.
- ☐ Tweezers.
- ☐ Ethanol (70-96%).
- ☐ Petri dishes.
- ☐ Several small containers where you will place the individuals of each species from each sampling site.
- ☐ Field digital weight scale.
- ☐ A dial or a digital caliper (0.01 mm) to measure the dung beetle traits.
- ☐ Mounting material.
- ☐ Precision digital scale (0.001 g).
- ☐ Oven (80 Cº).