I find a specimen of *Kaloplocamus ramosus* (Cantraine, 1835). What can I do ?

Quite a lot, actually, since so little is known about the life history and biology of this species.
To get the most out of your luck (this species is rarely seen), your efforts and the specimen you just found some method is needed.
By the way, we are also interested in specimens of ***Kaloplocamus acutus* Baba, 1949** for comparison purposes. This species has been spotted in the Indian and Pacific Oceans.

We are aware that to biologists or seasoned field professionals the following text (or parts of it) may look superfluous. Since we also wish to enroll amateur diver-naturalists in this project, we prefer to err on the 'too much details' side.

WARNING :
You are expected to inquire about and comply with all national and/or regional laws and regulations regarding the collection of marine fauna specimens at your location.
RBINS(*), DORIS(*) and the author of the present 'Protocol' waive any kind of responsibility if the aforementioned laws or regulations are not complied with.

The following 'to-do' list is an ideal sequence of actions. But, of course, we will be very happy with the fulfillment of any part of it.
At each step, take notes about what you see and do (it is never too much). The **"*K. ramosus* Field Sheet"** at the end of this Protocol will ensure that all important data are recorded in the field.
This will be part of the documentation file to be sent to us (see also § 6 Documentation).

If you plan to collect the specimen and/or its prey you will need between 150 and 200 ml of an approximately 96% ethanol solution without any additives. This is the only preservation and conservation product allowed because DNA analysis is planned. DNA analysis might help us explain the species' peculiar world distribution pattern and confirm if we are dealing with one very variable cosmopolitan species or with a number of similar looking species.
A 96% ethanol solution can normally be bought at a chemists if no lab is nearby or ready to help. Other products (formalin, for example) will alter the molecular composition of the tissues and prevent successful DNA analysis.

CAUTION: Ethanol is a very flammable product. Keep away from any heat source or flame.
And, do not drink it: industrial ethanol is toxic.

CAUTION: Ethanol dissolves color pigments immediately. Since *K. ramosus*'s colour patterns are quite variable, it is very important to photograph each specimen before immersing it in ethanol

1 – In situ (in the wild) when the specimen is alive

1.1 - Observe the behaviour of the specimen, measure its length or make some marks (on a finger or equipment) that will allow its determination later; note depth, water temperature, type of environment (crevice, under a stone or ledge, on the surface of a rock, etc.) ; night or day dive, etc.

1.2 - Photograph the specimen underwater in its environment from different angles (and also the said environment), then collect it (if you are allowed to).

1.3 - If the specimen was feeding (normally its prey will be a branching bryozoan), photograph and collect the prey for later identification (by someone familiar with the local fauna).

Photos of the specimen should ideally be taken at close range and at high resolution (5 to 10 Megapixels).

2 – At home or in the lab, with the specimen still alive

2.01 - Put the specimen in a seawater-filled container or small aquarium. When it's relaxed, measure it again and photograph it from different angles (for some photos, with a ruler beside it or under the container so it appears in the picture).

2.02 – If during collection it was seen feeding on a bryozoan and you also collected the bryozoan, put the latter in the container/aquarium along with the specimen and observe what happens. If a feeding event occurs, take pictures of it.

2.03 - Keep it, still alive, for a few days and try taking pictures of its sole (underside of the foot) when it is crawling on the container's or aquarium's walls.

2.04 - At night (or in the dark), prod it gently with a small plastic, glass or wooden rod: hopefully it will emit short bursts of bluish light. If your camera can take a series of photos in bursts, try and take some pictures of this bioluminescence. It is best to have the camera ready before starting the prodding.
Also try to record the number and duration of these light bursts until they stop.

2.05 - If the specimen deposits a ribbon of spawn (nudibranchs often do so when stressed by their capture), take note of the date and time of deposit. Then take 'whole ribbon' and close-up pictures (with as high a magnification as possible) of the egg ribbon and measure the diameter of the spiral as well as the width (= height) and length of the ribbon. If possible, take a picture or two with a ruler next to the spawn.

2.06 - If a dissecting microscope (x5 to x30 or x40) is available, carefully collect the spawn, put it under the binocular scope and take pictures of the egg capsules at different magnifications. A small handheld camera shooting through the eyepiece (ocular) can do the job quite well. If possible, measure the diameter and length of a number of egg capsules selected at random, (minimum 10, better 20). Note the magnification for each picture.

2.07 - Count the number of eggs (if there are several) per capsule (minimum 10, better 20 capsules).

2.08 - Count the number of capsules in the spawn (actually, estimate it by counting their number in a 1 mm wide transverse section, then multiplying by the measured length of the ribbon.

2.09 – If you notice some larvae hatching from the spawn, (this may happen from hours to days after spawning) note the date and time of the start. Also record if these larvae are swimming or if they immediately crawl on the bottom. Then, take some pictures and measure some (length and width). Note the magnification for each picture.

2.10 – Then, rinse the egg ribbon with some ethanol, put it in a separate small clean water-tight vial (with a threaded cap), immerse it in 96% ethanol and add a label (see § 5 - Labelling).
This vial should be sent together with the *K. ramosus* (or *K. acutus*) specimen.
This is of importance since, as yet, no one knows the species' type of larval development (the egg capsule's diameter can provide a clue). It may help us yo understand its worldwide distribution pattern.

2.11 - If the specimen's suspected prey was collected (branching bryozoan), photograph it at close-up range and under a dissecting microscope (x5 to x30 or x40) if it is available.
Then immerse it in a separate small clean water-tight vial (with a threaded cap) with 96% ethanol with proper labelling (see § 5 - Labelling) for later identification by someone familiar with the local fauna. This is also of importance since, as yet, no one knows *K. ramosus*'s prey species outside the Mediterranean Sea and the Gulf of Biscay (Europe).
This vial should be sent together with the *K. ramosus* (or *K. acutus*) specimen.

3 – When the specimen is dead

When all the above is done an other line of inquiry begins: preserve the specimen for future morphological and anatomical examination as well as for DNA analysis. This requires the use of a 96% ethanol solution without any additives. It can normally be bought at a chemists if no lab is nearby or ready to help.

At this point, either the animal has died naturally or it is still alive.
In the latter case, it will have to be narcotized (see § 4 – Narcotization).

After its death, the specimen should immediately be immersed in a small clean water-tight vial (with a threaded cap) with 96% ethanol in order to prevent decay. A proper label should be added to the vial (see § 5 - Labelling).

The volume of ethanol should be at least 5 times (5 to 10 times) the volume of the specimen. To my knowledge, an average *K. ramosus*'s volume is about 3-5 ml. The corresponding ethanol volume is about 25 ml. A clean (ethanol-rinsed) 30 ml vial will do.

Since the specimen will then start shedding water (because of the ethanol), this will dilute the solution. It is therefore important to replace the solution 2 to 3 times during the first 24 hours. If the ethanol is not readily available deep-freeze the specimen in sea water (with just enough water to cover it) until the ethanol is available. When thawing, discard as much water as possible.

4 – Narcotization

If you have to kill the specimen, it is best to narcotize it before doing so. It is more ethical and it will keep its relaxed posture, which makes it easier for future identification and examination.

Select one of the following methods:

4.1 – If magnesium chloride ($MgCl_2$) is available, put the animal in as little sea water as needed to cover it and wait for it to relax and extend. Then add a few drops (10) of a solution made of 7 g magnesium chloride in 93 g of fresh water (called a 7% solution).
A rounded teaspoon of $MgCl_2$ in a bit less than a liter of fresh water is OK.
Wait for an hour, then poke the specimen with a needle. If it does not move, it will probably be dead. If not, add a few more drops, wait an hour, etc. until the animal is dead. Then, put it immediately in a small clean water-tight vial with 96% ethanol, a proper label and proceed as outlined in § 3 (ethanol refreshing).

4.2 – If magnesium chloride is not available, first immerse the animal in sea water and put it in a refrigerator for a few hours until it is relaxed and extended. Then, deep freeze it, still in seawater, (an hour or two will do) until it's dead. Then, after thawing and rinsing in fresh water, put it immediately in a small clean water-tight vial with 96% ethanol, a proper label and proceed as outlined in § 3 (ethanol refreshing).

4.3 – If neither magnesium chloride nor a deep freezer is available, immerse the specimen in sea water, allow for relaxation, then gradually pour a small quantity of ethanol into the water and wait for about an hour. Poke the specimen with a needle. If it does not move, it will probably be dead. If not, add some more ethanol, wait an hour, etc. until the animal is dead. Then, put it immediately in a small clean water-tight vial with 96% ethanol, a proper label and proceed as outlined in § 3 (ethanol refreshing).

5 – Labelling

Without proper labelling, a sample is pretty useless. It is very important to know what is in the vial, where and when it was collected and by whom (in the event additional info is needed).
Use acid free paper or quality photocopying paper and a pencil. Put the label into the vial.
If you are not sure the paper is acid free, use what is available and attach it to the outside of the vial with transparent tape. Be generous with the tape to prevent loss of the label.

The notes and references to the photos you have taken will be recorded in detail in the **"*K. ramosus* Field Sheet"** (see § 6 – Documentation). If needed, additional pages are OK. Therefore the contents of the labels can be restricted to:

- **Field Identifier** of **specimen** : (unique reference on label, related documents, photos, etc.)	**KR_yyyy_your name** (**YYYY** = year) **KR_yyyy_your name_bryoz** or **_spawn** if it applies
- **Name of species**	**K. ramosus (Cantraine, 1835)** or **K. acutus Baba, 1955**
- **Collection date** (in YYYY-MM-DD format)	
- **Location** (ocean, country or island, dive-site) and/or (geographic coordinates)	See "*K. ramosus* Field Sheet" for details
- **Depth** (in meters)	
- **Collector** (full name)	**Coll.**: ---------
- If **Related stuff is linked** (photos, spawn, bryozoa etc.) mention what applies :	**'+ Docs' and/or 'Photos', 'spawn', bryozoa'** etc.

If more than one specimen is collected at the same site, add a sub-number at the end of the identifier for each specimen and mention them in the **"*K. ramosus* Field Sheet"**.
If more than one specimen is collected on the same day but at different sites, fill-in one **"*K. ramosus* Field Sheet"** per location, each with its own 'Field Identifier'.

6 – Documentation ("*K. ramosus* Field Sheet")

All the sample-related information you collected (location name and its geographic coordinates, depth, prey, surroundings, measurements, notes you took, etc.) can be recorded in the **"*K. ramosus* Field Sheet"** hereunder.

Your name, e-mail address and any restrictions you may wish to put on the future use of your material should also be mentioned there.

Once the 'Field Sheet' is completed and signed, scan it (this is preferred) or take a sharp photo of it. The scan (or photo) of the **"*K. ramosus* Field Sheet"**, along with any other information and the photos you wish to share with us should be sent in digital form, by e-mail, to both the following adresses:

1- JEMU (c/o Gontran Sonet / RBINS) – e-mail : gontran.sonet(at)naturalsciences.be
2- Alex Vanhaelen – e-mail : Kramosus.project(at)hotmail.com

7 – Packaging and forwarding

The vial (or vials) should then be carefully packaged in such a way that it will not leak during transport and sent, inclusive a paper copy of the "Field sheet", as soon as possible to :

Joint Experimental Molecular Unit (JEMU) – K.ramosus project
c/o Gontran Sonet - Operational Division Phylogeny and Taxonomy
Royal Belgian Institute for Natural Sciences (RBINS)
Rue Vautier street, 29
B- 1000 Brussels
BELGIUM (Europe)

Your specimen will be stored at RBINS, in a –80°C deep-freezer, waiting for enough company, from as many places around the world as possible, to allow a full DNA study.

If you want to know more about this species have a look at
- DORIS link (in French) http://doris.ffessm.fr/fiche2.asp?fiche_numero=3812
- Sea Slug Forum : http://www.seaslugforum.net/showall/kaloramo
- Sea Slug Forum : http://www.seaslugforum.net/showall/kaloacut

We wish to thank you very much for your help in our attempt to unravel *K. ramosus*'s secrets.

N.B. This document owes enormously to Virginie Winant (RMCA), Gontran Sonet (JEMU/RBINS) and Cory Pittman (www.seaslugsofhawaii.com).

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RBINS : Royal Belgian Institute for Natural Sciences, Brussels, Belgium. (www.naturalsciences.be)
RMCA : Royal Museum for Central Africa, Tervuren, Belgium (www.africamuseum.be)
DORIS : Données d'Observations pour la Reconnaissance et l'Identification de la faune et de la flore Subaquatiques. (http://doris.ffessm.fr/)

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"*K. ramosus* Field Sheet"

A scan or sharp photos of this completed document should be sent along with the digital file to
1- JEMU (c/o Gontran Sonet / RBINS) – e-mail : gontran.sonet(at)naturalsciences.be
2- Alex Vanhaelen – e-mail : Kramosus.project(at)hotmail.com

Project : Kramosus project		**RBINS's ref.: I.G.**	&	**RBINS's INV. Nbr :**

Field Identifier (as on label): (YYYY = year) If the specimen is a **K. acutus**:	**KR_YYYY_your name** (or part of it) for the specimen **KR_YYYY_your name_spawn** for a spawn if it applies **KR_YYYY_your name_bryoz** for suspected prey if it applies **KA_YYYY_your name** (or part of it) for the specimen etc.

Collector : (your last name) your 'christian' name: (or its equivalent)	- -
Full postal address (included country & area code) : e-mail address:	

Collection data

Collection date (in YYYY-MM-DD format) :	
Depth (in meters) :	
Day-light or night dive:	

Location (administrative names)

Ocean :	**Sea** :	**Continent** :

Country (full name) :	**Country** (ISO country code) :
State or Province :	
District (park, county, lake, river):	**Municipality** :

Exact site (specific locality including a site name, how many kilometers and compass direction from the nearest major specific map location [e.g. town, mountain peak, lake, specific park or road network, etc.] - All distances should be presented in metric units.)

Geographic coordinates (must be in "degrees.decimaldegrees" format. Don't forget to set your GPS receiver to the default datum setting of World Geodetic System 1984 (WGS84). Record data with at least two digits to the right of the decimal point for better precision (e.g. 45.837).
Note that "south" latitudes and "west" longitudes convert to ***negative*** decimal numbers.

Latitude (north-south)	
Longitude (east-west)	

Ecology (any information related to the sampling site [e.g. biotope description, salinity, water temperature, nature of substrate, currents, where was the specimen found--under a stone, in a hole or cave, etc.])

Collection data (how was the specimen collected and handled ?)

Taxonomy

Taxon name	**Genus** : **species** :
Author (name, year)	
Identified by (name, date)	
Identified with (source reference) (guide, book, paper, website)	

Photo documentation (photo naming: photo number and 'text' by photographer) [*e.g.* – photo number_KR_collector's name & initials_text] If needed, make a separate list.)

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Miscellaneous

Spawn linked to sample ? (NO or YES + label identifier) : **Bryozoa (prey) linked to sample ?** (NO or YES + label identifier) : **Other information** :

Restrictions on the use of your material
(which restrictions, if any, do you want to put on the use of your material ? If any, give a detailed list of the material and the linked restriction.)

Date : ----/----/-------
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Your signature :