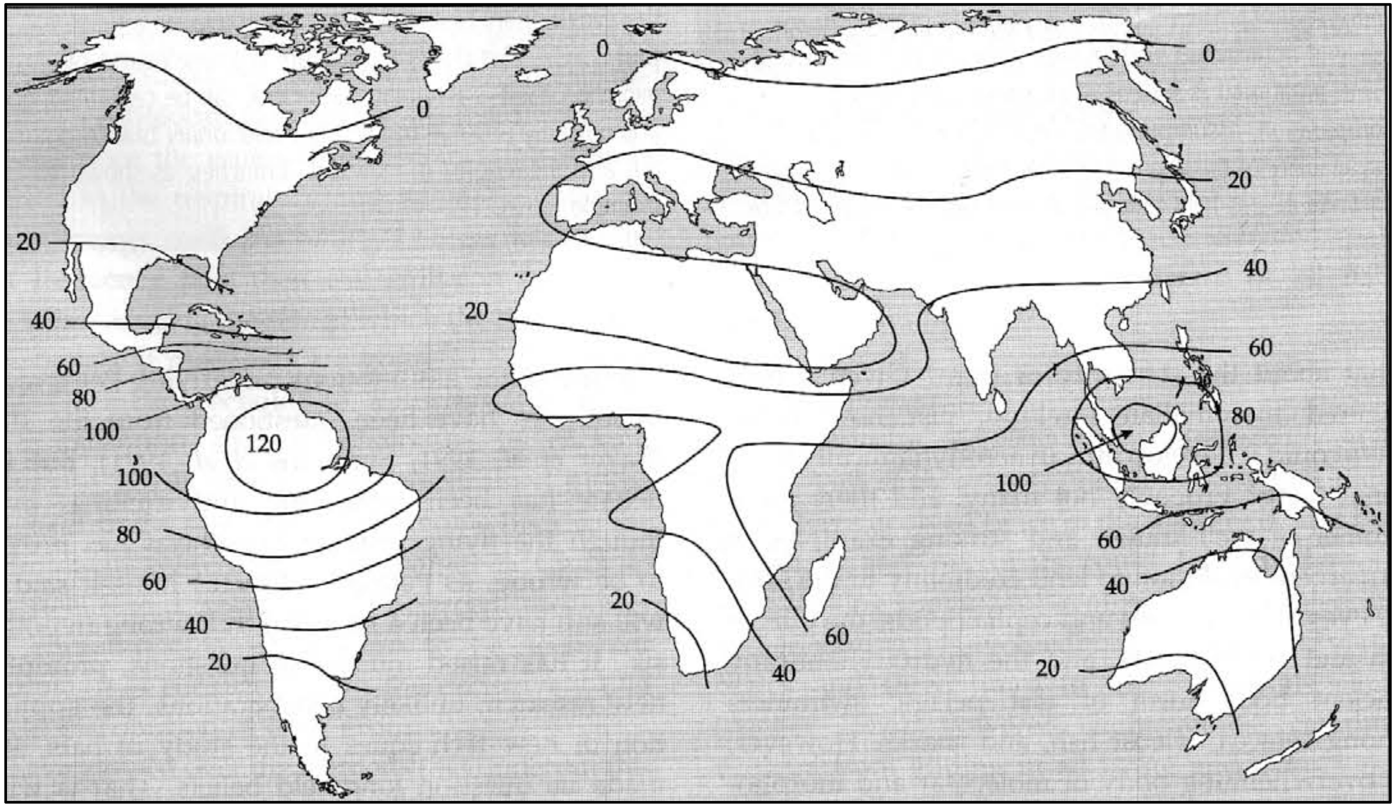
Bat Taxonomy and Identification

Benjamin Lee

Global distribution of bats



The number of bat species in 500 km^2 quadrats in different parts of the world. (Findley 1993)

Classification

20% of about 5,420 species of mammals are bats (Altringham 2011)

Traditionally, two sub-orders:

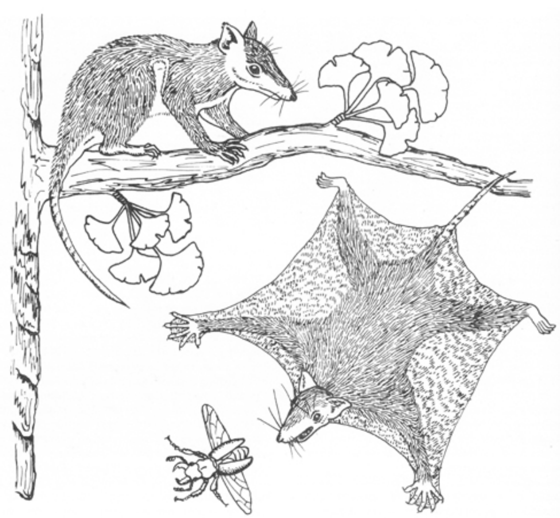
- Megachiroptera (megabats)
  - "large" and feed on fruit, nectar and blossom
  - well-developed vision and olfaction
  - do not use echolocation except *Rousettus*
- Microchiprotera (microbats)
  - most are "small" with a wingspan of 30cm
  - diverse diet groups but mostly insectivorous

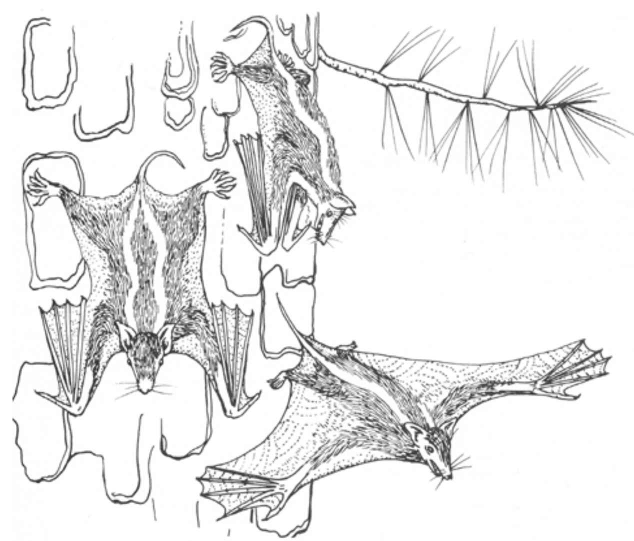
Recent molecular studies

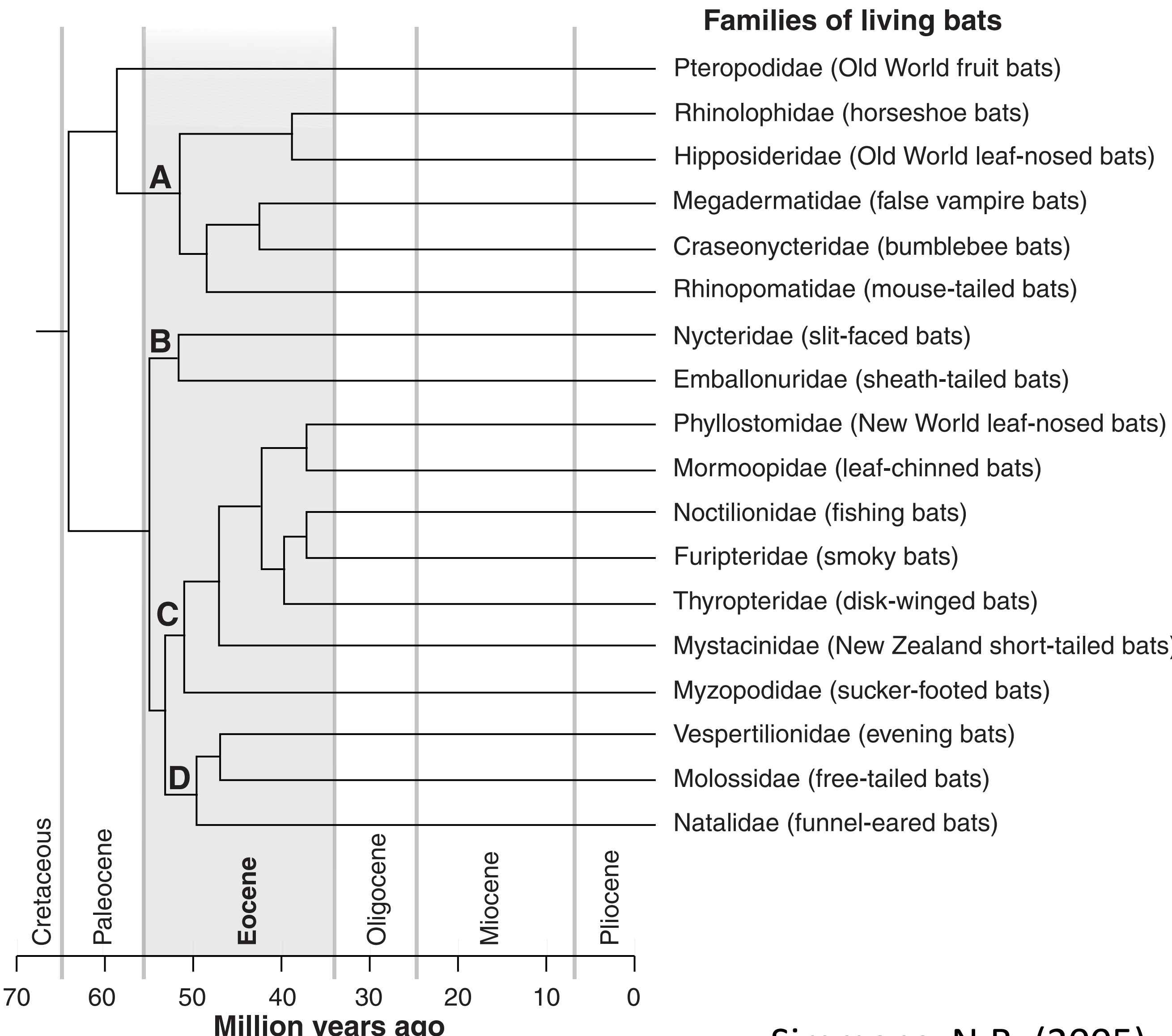
- Invalidates previous classification
- Molecular time-scale - reconstruct evolutionary pathways of bats
- Evolved from a common ancestor of the mammal group
- Yinptero and Yango diverged early – 64 mya
- Pteropodidae and Rhinolophoidea – split 58 mya

Recent molecular studies

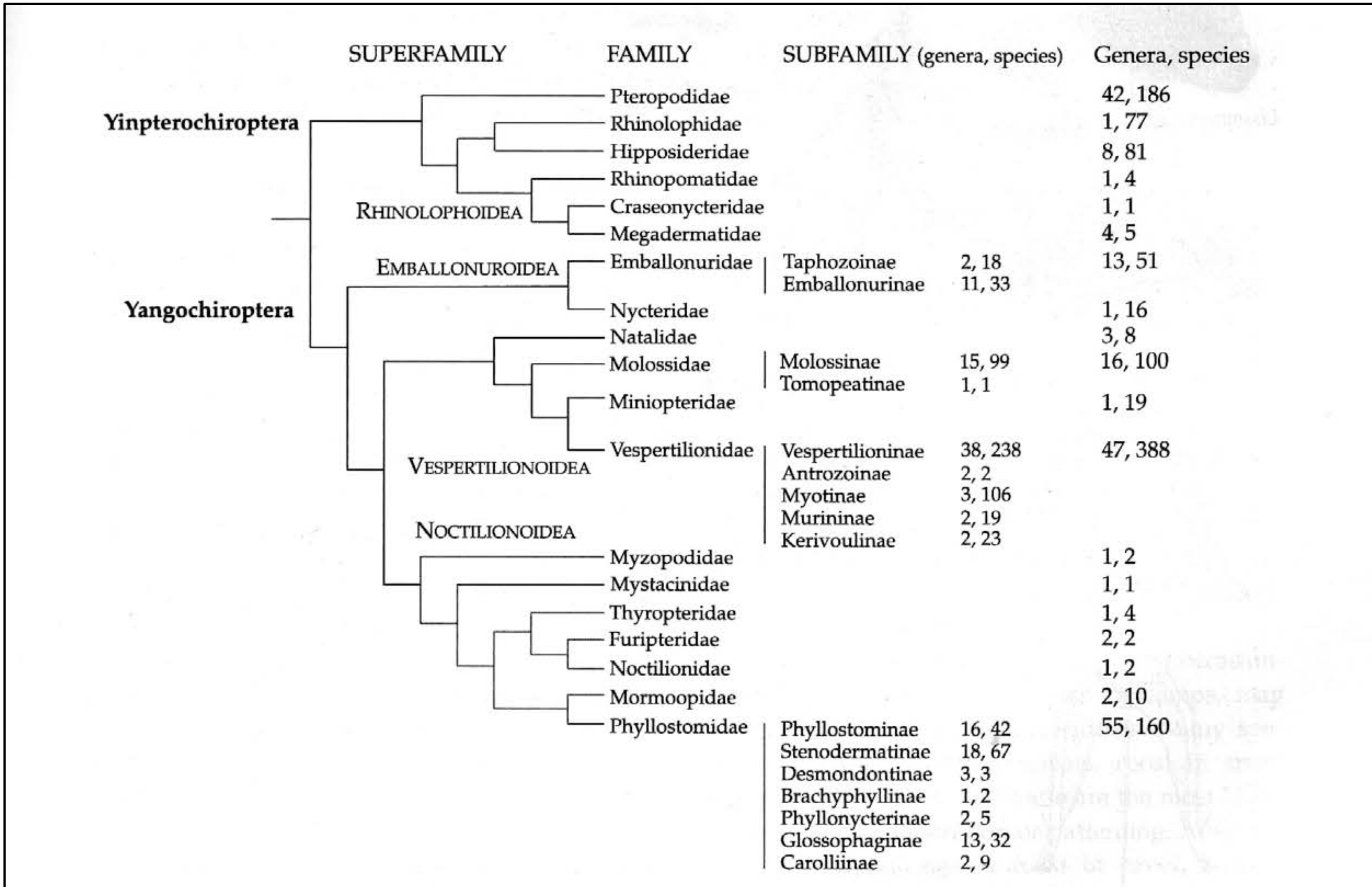
- **Yinpterochiroptera** – Rhinolophoidea + Pteropodidae
- **Yangochiroptera** – Emballonuroidea + Noctilionoidea + Vespertilionoidea
- Split 52-50 mya during global warming and peak plant and insect diversity
- Improvement in echolocation and wing morphology



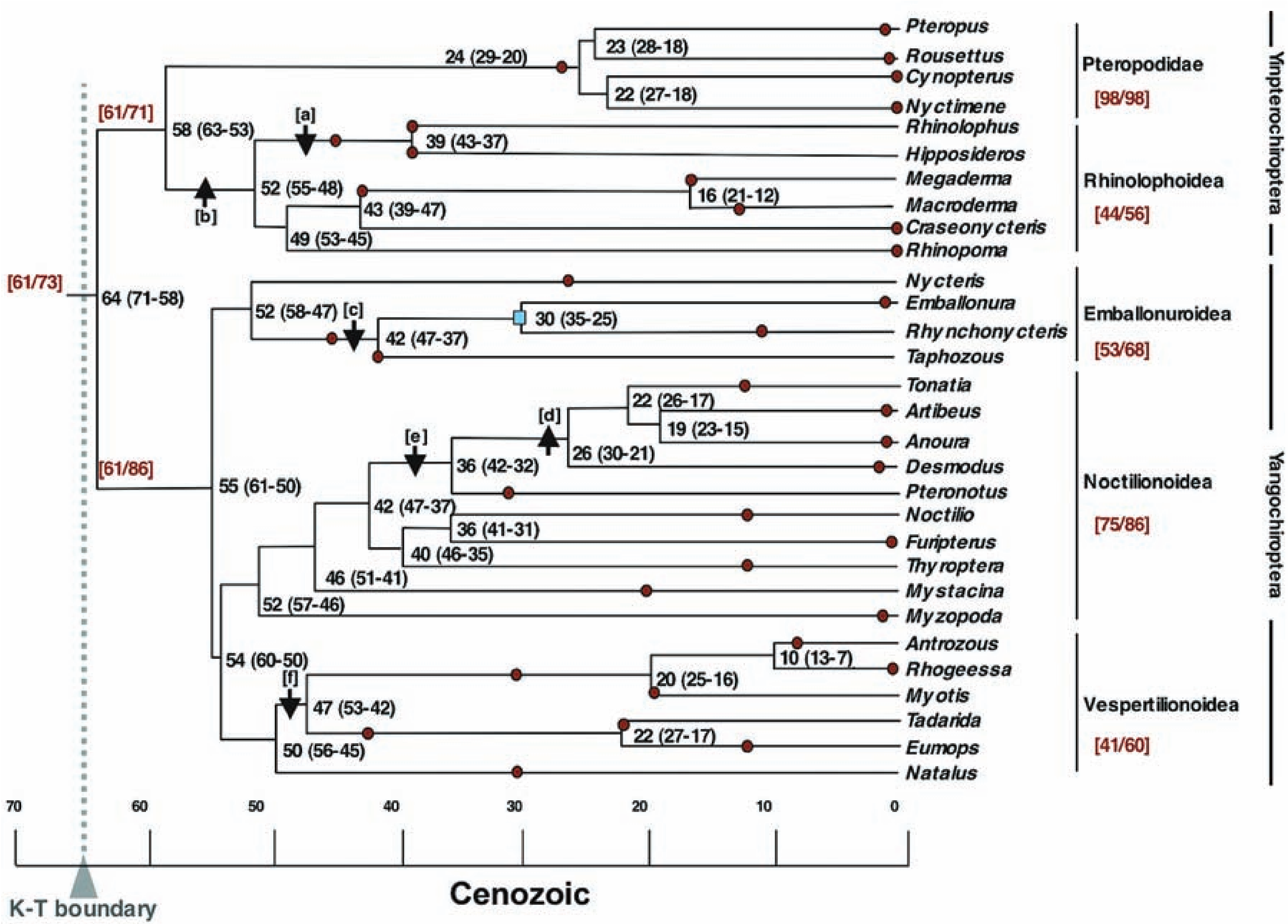




Simmons, N.B. (2005)



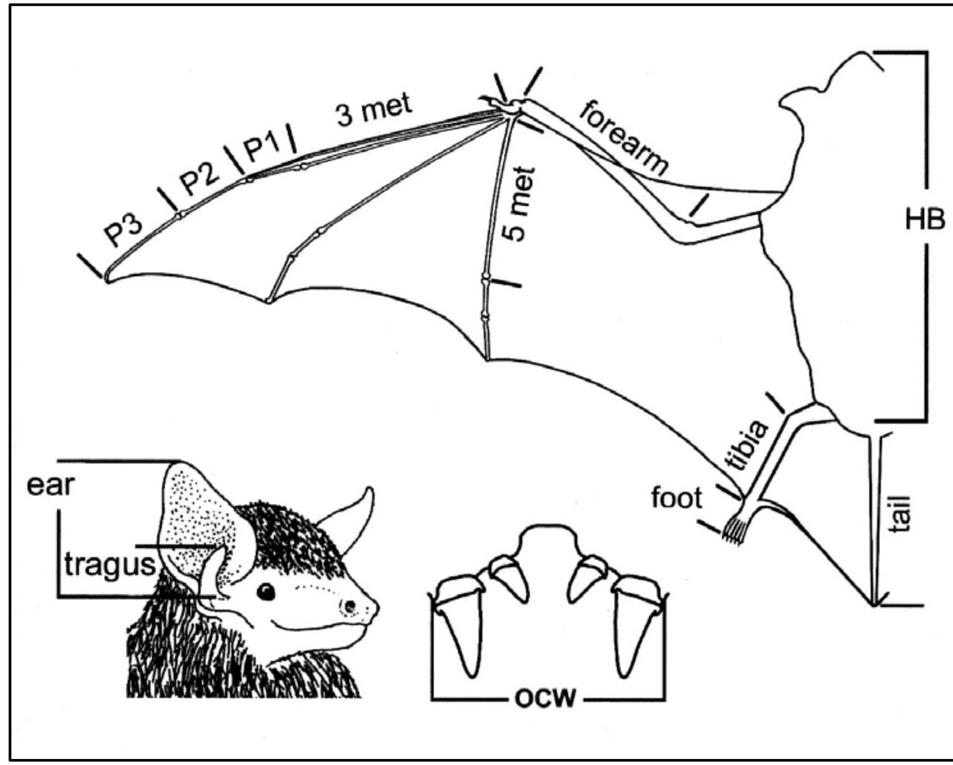
An evolutionary tree for the bats to sub-family level based on Teeling *et al.* (2005)



Teeling et al. (2005)

Identification – morphological approach

- Bat species – distinguished based on morphological characteristics
  - Body shape, size and weight
  - Forearm length
  - Ear and tragus
  - Fur colour
  - Teeth and skull



Churchill, S. (2008)

- Statistical analysis of large numbers of measurements – repeatability and more credible

3

Field Guide to the Bats of Krau Wildlife Reserve

Key to the Bats of Peninsular Malaysia

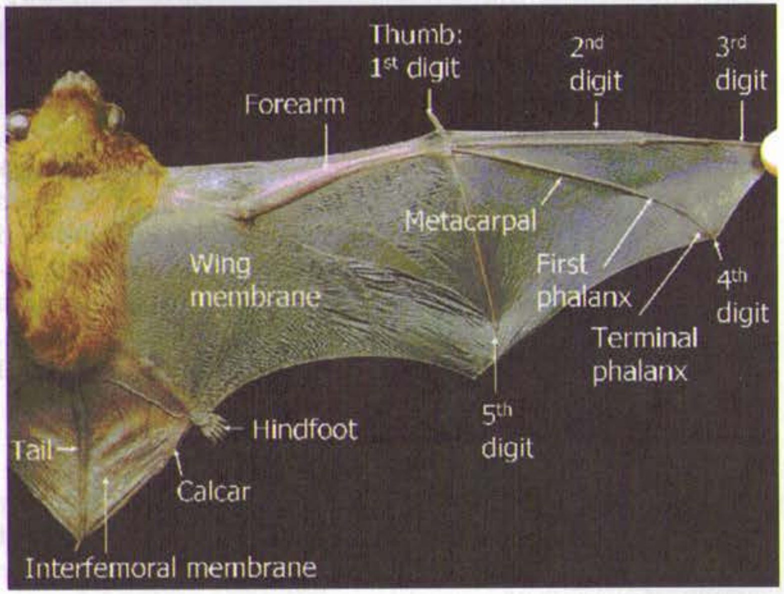


FIGURE 3.1 Dorsal view of *Scotophilus kuhlii* to show parts of a bat (Photo Wood)

Key to Families

1 Claw on 2nd digit (except *Eonycteris*); simple ears with outer margins forming an unbreakable ring; dog- or fox-like face; FA 40-220 mm PTEROPODIDAE

No claw on 2nd digit; ears often complex with tragus; face not dog-like 2

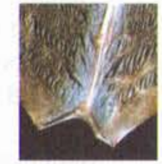


FIGURE 3.2

2 Tail ends in a T-or V-shaped cartilage enclosed in interfemoral membrane (Figure 3.2); deep slit in face, ears large, separated at base NYCTERIDAE

Tail does not end in a T-shaped cartilage, no deep slit in face 3

FIGURE 3.3

3 Tail extends for at least half its length beyond the end of interfemoral membrane (Figure 3.3) MOLOSSIDAE

Tail is shorter than the interfemoral membrane and protrudes from the middle of the dorsal side of the membrane (Figure 3.4) EMBALLONURIDAE

FIGURE 3.4

Tail, if present, is fully enclosed in interfemoral membrane (tip may just appear to extend beyond, but is within the membrane) (Figure 3.5) 4

FIGURE 3.5

4 Plain nose VESPERTILIONIDAE

Leaf-like structure on nose and face 5

5 Tragus long and bifurcate (Figure 3.6); large rounded ears joined across top of head; upper incisors lacking MEGADERMATIDAE

Tragus simple; ears not joined across top of head; upper incisors present 6

FIGURE 3.6

Kingston *et al.* (2006)

Identification – morphological approach

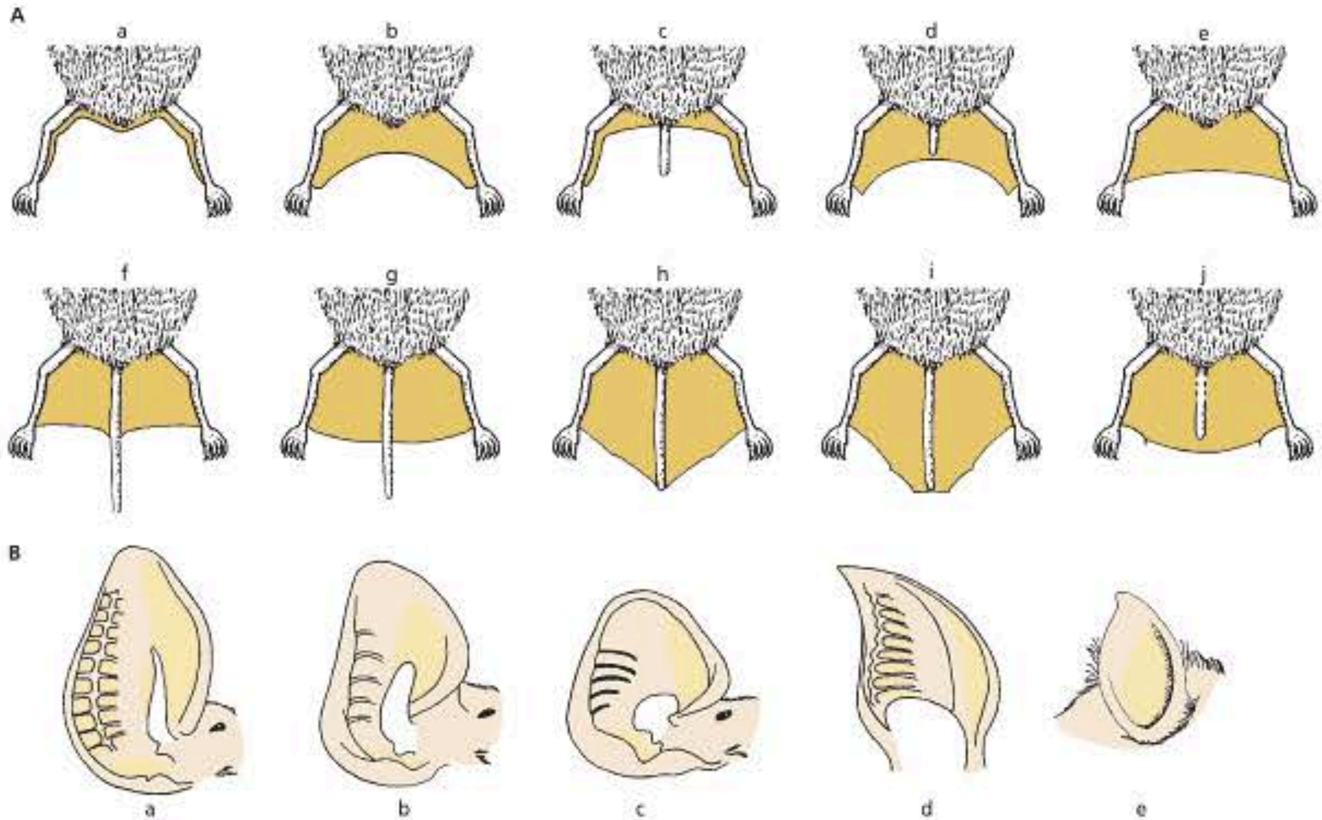


Figure 13.2 Variation in the uropatagium, tail, external ear, and tragus of representative families of bats. (A) (a) Fruit bats (Pteropodidae) in the genus *Pteropus*, as well as some New World leaf-nosed bats (Phyllostomidae); (b) New World leaf-nosed bats; (c) tube-nosed fruit bats in the genera *Nyctimene* and *Paranyctimene* (Pteropodidae); (d) New World leaf-nosed bats; (e) hog-nosed bat (Craseonycteridae), with extensive uropatagium but no external tail; (f) mouse-tailed bats (Rhinopomatidae) with long, thin tail free of the uropatagium; (g) free-tailed bats (Molossidae) with about one-half the tail free of the uropatagium; (h) common bats (Vespertilionidae), similar to tails in horseshoe bats (Rhinolophidae) and false vampire bats (Megadermatidae); (i) slit-faced bats (Nycteridae) with T-shaped tip of the tail; (j) sac-winged bats (Emballonuridae), fishing bats (Noctilionidae), and moustached bats (Mormoopidae) have a tail that protrudes through the uropatagium. (B) Representative sizes and shapes of the external ears and tragus in bats. (a–c) Variation in size and shape of the ear and tragus in vespertilionids; (d) antitragus in a rhinolophid; (e) continuous inner ear margin without tragus or antitragus in a pteropodid. *Adapted from Hill and Smith, 1984,* Bats: A Natural History, *British Museum of Natural History.*

Identification – genetic approach

- Revolution in taxonomy

- Genetic analysis
  - Clarify occurrences of species / species boundaries (e.g. *Myotis adversus*)
  - Discriminate species using DNA barcodes in highly diverse tropical bat assemblages (e.g. cytochrome *c* oxidase subunit 1 gen in Guyanan bats)

- Application of genetic methods – more stable and scientifically based classification

Identification – acoustic approach

- Requires automatic classifiers or call libraries
- Good ecological knowledge of foraging behaviours in different habitat types

Examples:

- *Hipposideros bicolor* – 2 phonic types

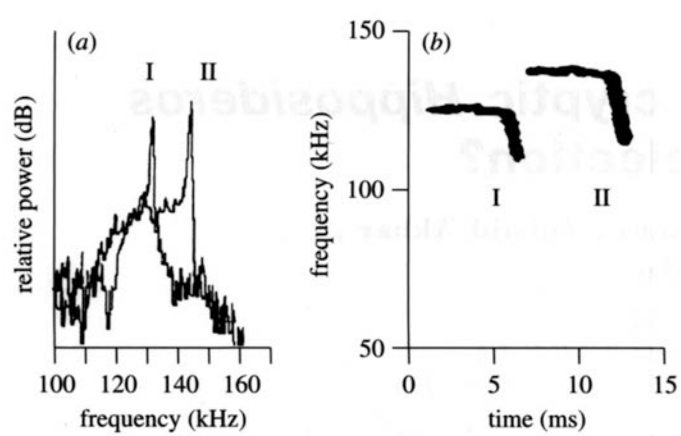
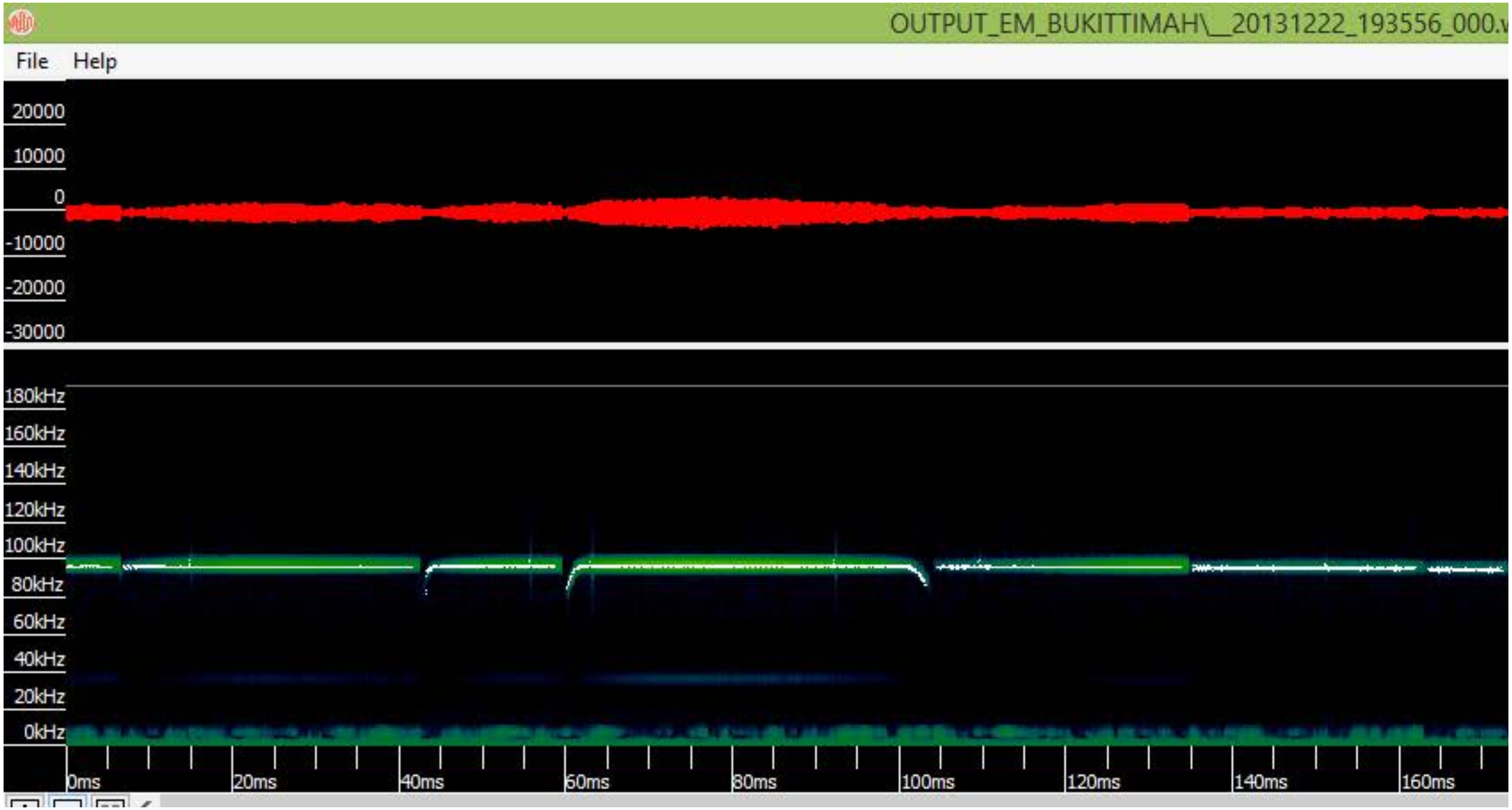


Figure 1. Echolocation calls of two phonic types of *H. bicolor*. (*a*) Power spectra and (*b*) sonagrams. Call I had a frequency of maximum energy of 131 kHz and call II a frequency of maximum energy of 142 kHz.

Kingston, T., *et al.* (2001)

Identification – acoustic approach

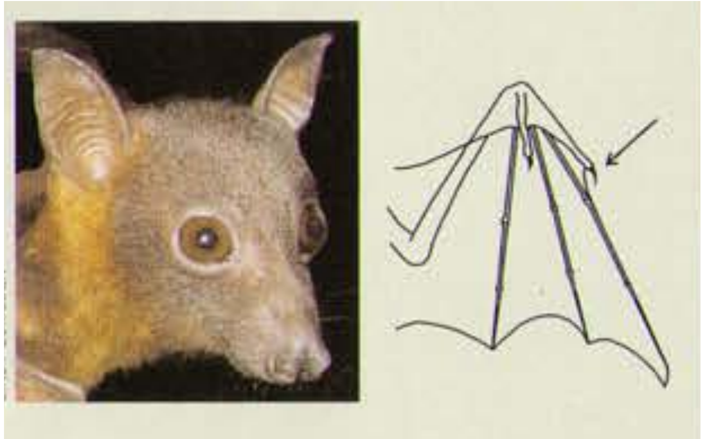
- *Rhinolophus lepidus* – constant frequency (CF) calls



97 kHz

Pteropodidae (Old World fruit bats)

- Simple ears with outer margins forming an unbroken ring
- Dog- or fox-like face
- FA 40-220 mm
- Tail short or lacking, when present not involved with membrane
- Interfemoral membrane narrow
- Simple cusps



Kingston *et al.* (2006)

Emballonuridae (sheath-tailed bats)

- Small to large
- Variable ears, often joined anteriorly at the base over the forehead
- Small or moderate tragus
- No noseleaf
- Glandular pouch or beard may be present on throat
- Tail partly enclosed in moderate tail membrane, its terminal part projecting from the upper surface

Kingston *et al.* (2006)

Nycteridae (slit-faced bats)

- Medium to large
- Very long ears separated at base
- Short rounded tragus
- Long tail with T- or V-shaped tip fully enclosed in interfemoral membrane
- Face has deep groove in middle bordered by leaf-like flaps of skin
- *Nycteris* is the sole genus

Kingston *et al.* (2006)

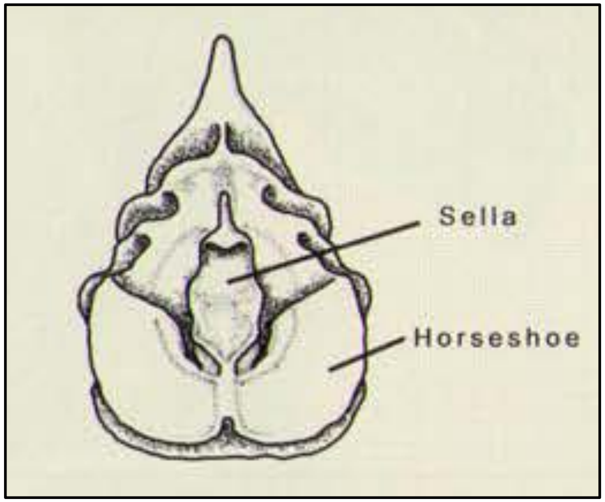
Megadermatidae (false vampire bats)

- Medium to large
- Large erect noseleaf
- Large ears joined across the top of the head by a band of integument
- Tragus long and forked (bifurcated)
- Very short tail, not visible externally, but interfemoral membrane well developed

Kingston *et al.* (2006)

Rhinolophidae - characteristics

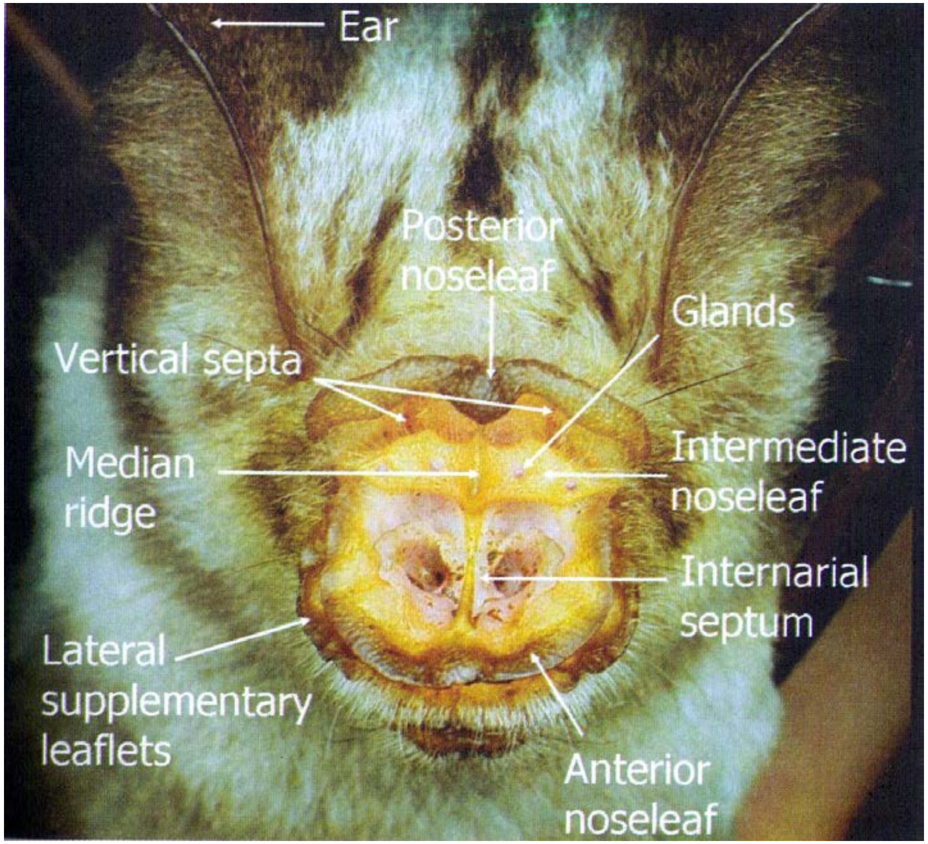
- Small to medium size
- Elaborate noseleaf: anterior section is rounded and roughly horse-shoe-shaped; behind the nostril is a raised portion – the sella; behind this is a posterior noseleaf which rises to a long, lancet-shaped point
- Ears large with prominent anti-tragus



Churchill, S. (2008)
Kingston *et al.* (2006)

Hipposideridae (Old World leaf-nosed bats)

- Small to moderately large
- Elaborate noseleaf but less extensive (c.f. with rhinolophids). Anterior noseleaf is rounded and horse-shoe-shaped; median leaf is a low cushion-like structure expanded laterally **without a sella**; posterior leaf is low and rounded, usually divided by vertical septa into several pockets
- Low antitragus
- Eyes very small



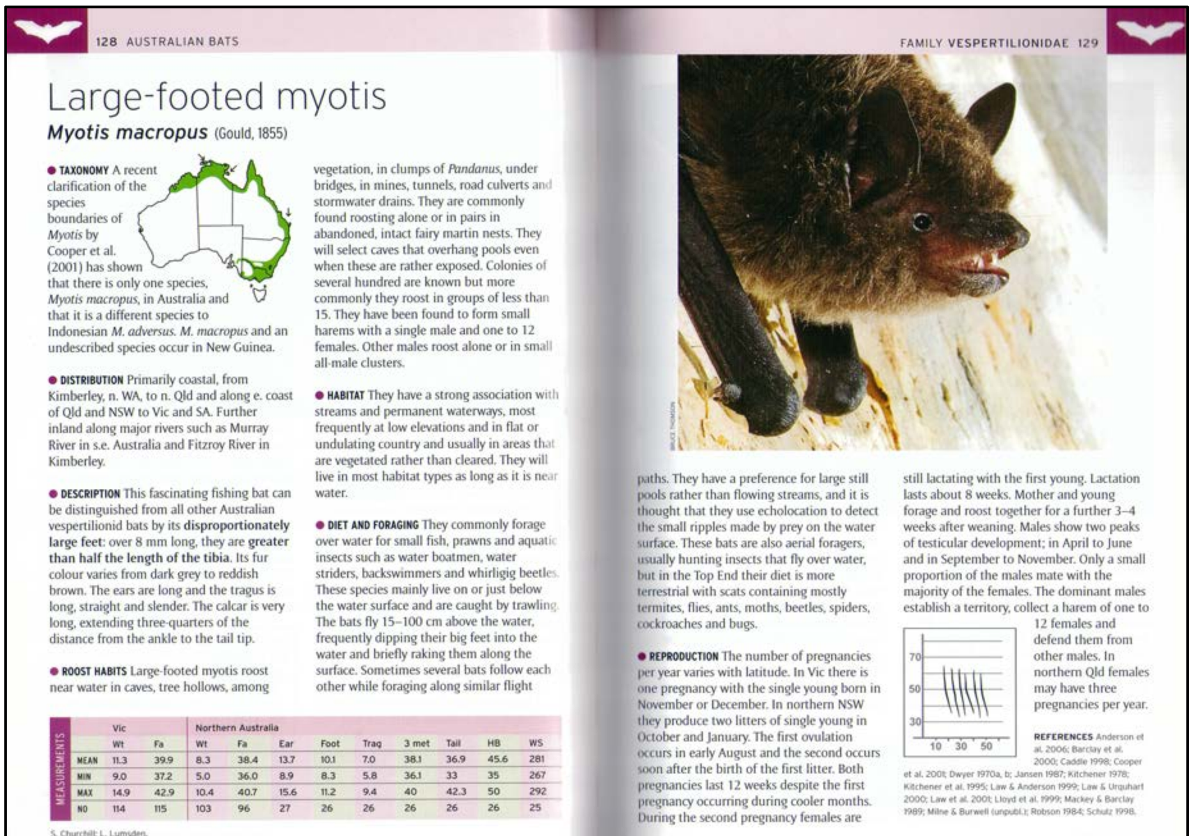
Kingston *et al.* (2006)

Vespertilionidae (Evening bats)

- Nose simple without noseleaf (rudimentary in Nyctophilus and Pharotis)
- Ears small to large with a well-developed tragus
- Tail long and completely enclosed in the interfemoral membrane

Further identification help

- Experts in the bats of that region
- Published literature – checklists, keys, field guides, apps
- Bat collection in museums



Large-footed myotis

Myotis macropus (Gould, 1855)

TAXONOMY A recent clarification of the species boundaries of *Myotis* by Cooper et al. (2001) has shown that there is only one species, *Myotis macropus*, in Australia and that it is a different species to Indonesian *M. adversus*. *M. macropus* and an undescribed species occur in New Guinea.

DISTRIBUTION Primarily coastal, from Kimberley, n. WA, to n. Qld and along e. coast of Qld and NSW to Vic and SA. Further inland along major rivers such as Murray River in s.e. Australia and Fitzroy River in Kimberley.

DESCRIPTION This fascinating fishing bat can be distinguished from all other Australian vespertilionid bats by its **disproportionately large feet**: over 8 mm long, they are **greater than half the length of the tibia**. Its fur colour varies from dark grey to reddish brown. The ears are long and the tragus is long, straight and slender. The calcar is very long, extending three-quarters of the distance from the ankle to the tail tip.

ROOST HABITS Large-footed myotis roost near water in caves, tree hollows, among vegetation, in clumps of *Pandanus*, under bridges, in mines, tunnels, road culverts and stormwater drains. They are commonly found roosting alone or in pairs in abandoned, intact fairy martin nests. They will select caves that overhang pools even when these are rather exposed. Colonies of several hundred are known but more commonly they roost in groups of less than 15. They have been found to form small harems with a single male and one to 12 females. Other males roost alone or in small all-male clusters.

HABITAT They have a strong association with streams and permanent waterways, most frequently at low elevations and in flat or undulating country and usually in areas that are vegetated rather than cleared. They will live in most habitat types as long as it is near water.

DIET AND FORAGING They commonly forage over water for small fish, prawns and aquatic insects such as water boatmen, water striders, backswimmers and whirligig beetles. These species mainly live on or just below the water surface and are caught by trawling. The bats fly 15–100 cm above the water, frequently dipping their big feet into the water and briefly raking them along the surface. Sometimes several bats follow each other while foraging along similar flight paths. They have a preference for large still pools rather than flowing streams, and it is thought that they use echolocation to detect the small ripples made by prey on the water surface. These bats are also aerial foragers, usually hunting insects that fly over water, but in the Top End their diet is more terrestrial with scats containing mostly termites, flies, ants, moths, beetles, spiders, cockroaches and bugs.

REPRODUCTION The number of pregnancies per year varies with latitude. In Vic there is one pregnancy with the single young born in November or December. In northern NSW they produce two litters of single young in October and January. The first ovulation occurs in early August and the second occurs soon after the birth of the first litter. Both pregnancies last 12 weeks despite the first pregnancy occurring during cooler months. During the second pregnancy females are still lactating with the first young. Lactation lasts about 8 weeks. Mother and young forage and roost together for a further 3–4 weeks after weaning. Males show two peaks of testicular development; in April to June and in September to November. Only a small proportion of the males mate with the majority of the females. The dominant males establish a territory, collect a harem of one to 12 females and defend them from other males. In northern Qld females may have three pregnancies per year.

MEASUREMENTS	Vic		Northern Australia								
	Wt	Fa	Wt	Fa	Ear	Foot	Traq	3 met	Tail	HB	WS
MEAN	11.3	39.9	8.3	38.4	13.7	10.1	7.0	38.1	36.9	45.6	281
MIN	9.0	37.2	5.0	36.0	8.9	8.3	5.8	36.1	33	35	267
MAX	14.9	42.9	10.4	40.7	15.6	11.2	9.4	40	42.3	50	292
NO	114	115	103	96	27	26	26	26	26	26	25

S. Churchill; L. Lumsden.

REFERENCES Anderson et al. 2006; Barclay et al. 2000; Caddle 1998; Cooper et al. 2001; Dwyer 1970a, b; Jansen 1987; Kitchener 1978; Kitchener et al. 1995; Law & Anderson 1999; Law & Urquhart 2000; Law et al. 2001; Lloyd et al. 1999; Mackey & Barclay 1989; Milne & Burwell (unpubl.); Robson 1984; Schulz 1998.

Churchill, S. (2008)

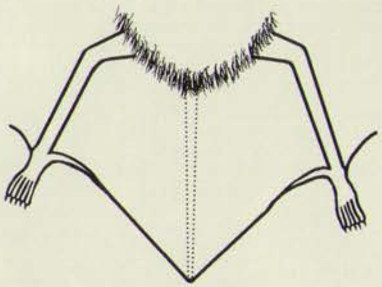
Key to bat families

1a Tail membrane absent or, if present, not joined between legs; claws on thumb and second finger of wing (except *Dobsonia* which has furless back); eyes large, head dog-like; no fleshy nose-leaf; navigates using eyesight.
→ ● **Family Pteropodidae** (page 62)

LINDY LUMSDEN

1b Tail membrane joined between legs; no claw on second finger of wing; eyes relatively small (except ghost bat); may have fleshy nose-leaf and/or complex folds; navigates using echolocation. → **2**

2a Tail fully enclosed within the tail membrane *or* tail is absent. → **3**



2b Tail not fully enclosed in tail membrane → **7**

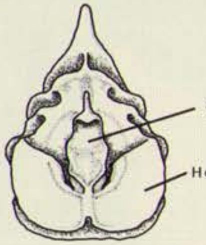
3a Large elaborate nose-leaf covering large portion of face. → **4**

3b Nose-leaf absent or small simple ridge on muzzle. → **6**

4a No tail but full tail membrane; large bat; prominent eyes; large ears joined above head; forked tragus of ear.
→ ● **Family Megadermatidae** (page 82)

4b Tail present; eyes small; tragus absent. → **5**

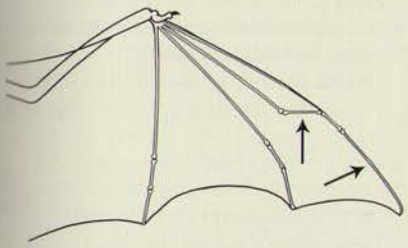
5a Nose-leaf covers most of face; lower leaf a distinct horseshoe shape; large projection (sella) protrudes from the centre of the nose-leaf and the upper leaf is pointed; toes with three joints each.
→ ● **Family Rhinolophidae** (page 84)



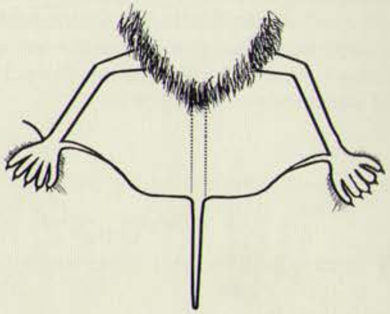
5b Nose-leaf less extensive; a flattened square or oval disk; no sella protruding from the centre, but small club-shaped structures may be present; the upper leaf is square or rounded; toes with two joints each.
→ ● **Family Hipposideridae** (page 90)

6a Terminal phalanx of the third digit of wing is a similar length to the second phalanx.
→ ● **Family Vespertilionidae** (page 104)

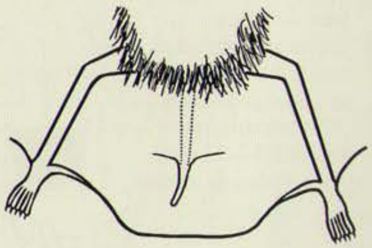
6b Terminal phalanx of the third digit of wing is at least three times the length of the second phalanx.
→ ● **Family Miniopteridae** (page 179)



7a Substantial portion of tail extends beyond the tail membrane.
→ ● **Family Molossidae** (page 188)



7b End of tail projects through the upper surface of tail membrane into a sheath of skin.
→ ● **Family Emballonuridae** (page 205)



Churchill, S. (2008)

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The biology of bats and their function as reservoir hosts

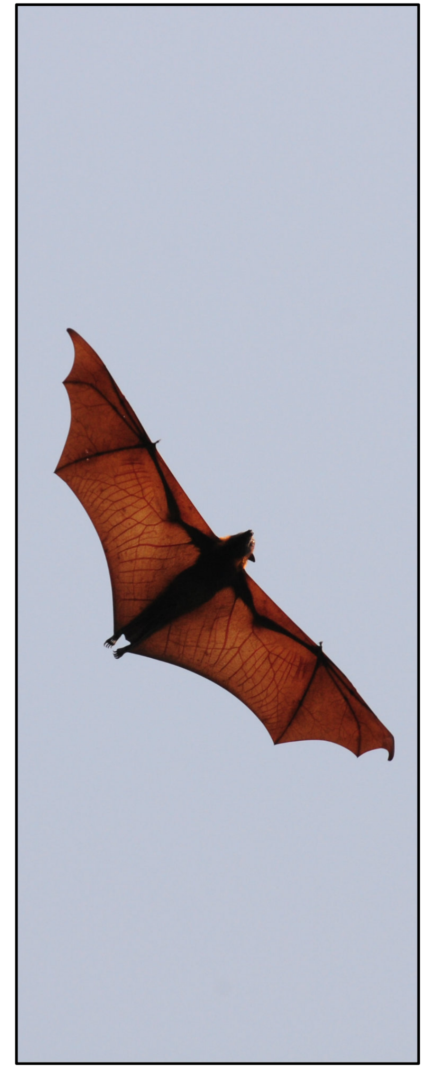
Benjamin Lee

Evolution and phylogeny

- Highly incomplete fossil record
- Nuclear genes - > bats originated in the Eocene period (50-52 mya)
- Bats evolved early, changed relatively little
- Long history of co-speciation with viruses (Gould 1996; Badrane and Tordo 2001)
- Order Chiroptera – meets the criterion of large group sizes and hence functions as potential animal reservoirs (AR).

Ability of flight

- Unique among mammals – true powered flight
- Fly daily to forage, and longer distances for seasonal migration
- *Tadarida brasiliensis mexicana* flies ≥ 800 miles during annual migration
- *Pteropus vampyrus* (maximum distance in 2 hrs = 130 km)
- *Eonycteris spelaea* (travels up to 60 km from roost and possible virus transmission when feeding)



Ability of flight

- Flying elevates metabolism and body temperature
- Selective force for co-existence with viral parasites
- Host-virus interaction = diverse zoonotic viruses
- Bat viruses more tolerant of a fever response and less virulent to hosts
- Hypothesis that needs testing

Bat Flight and Zoonotic Viruses

Thomas J. O'Shea, Paul M. Cryan, Andrew A. Cunningham, Anthony R. Fooks, David T.S. Hayman, Angela D. Luis, Alison J. Peel, Raina K. Plowright, and James L.N. Wood

O'Shea *et al.* (2014) EID 20: 741-745

Torpor and hibernation

- **Torpor** : the state in which an animal allows its body temperature to fall below its active, **homeothermic** level.
  – Drop in temperature is controlled and does not fluctuate with air temperature
  – If it gets too cold, some stored fuel is burnt to keep itself from becoming too cold

- **Hibernation** is an extension of torpor – days, weeks or months, seasonal basis, in response to a prolonged fall in temperature or reduction in food supply

- Bats do not usually practise continuous hibernation

Torpor and hibernation

- What is the impact of torpor and hibernation on maintenance of viral infections in bats?
- Viruses may over-winter in bats and infected bats may shed viruses such as lyssaviruses or flaviviruses for extensive periods without showing evidence of disease
- Big and little brown bats infected with JEV and subjected to temp (8-24°C) encountered during hibernation
  - Maintained viremias for 95-108 days
  - Virus titers in the blood of bats at 24°C = peak viral titers at temp at which bats were active

Long life span

- Extreme longevity + persistent infections with viruses -> maintain the virus and transmit
- Bats of the suborder Microchiroptera have life spans > 25 years (35 years for a little brown bat!)
- Bats become infected with viruses and infectivity lasting for months and years -> significant impact on the basic reproductive number of infection (R_o)
- Persistent viral infection in long-lived bats -> increase the potential for both intra- and interspecies viral transmission

Population size and roosting behaviour

- Great population densities and crowded roosting behaviour -> increased likelihood of viral transmission
- One of the most abundant mammals and widely distributed
- Mexican free-tailed bats – packed in caves at 300 bats per ft^2
- Airborne transmission of rabies virus – droplets of excreta or small particle aerosol

Bat population structure

- Demographic and spatial structuring of bat populations -> sufficiently variable to offer opportunities for viruses to be maintained
- Migratory and non-migratory populations serve as a mixing vessel for viruses
- Bat populations may be panmictic or exists as metapopulations -> seasonal virus transmission annual outbreaks of viral diseases, and periodic outbreaks among spatially discrete populations

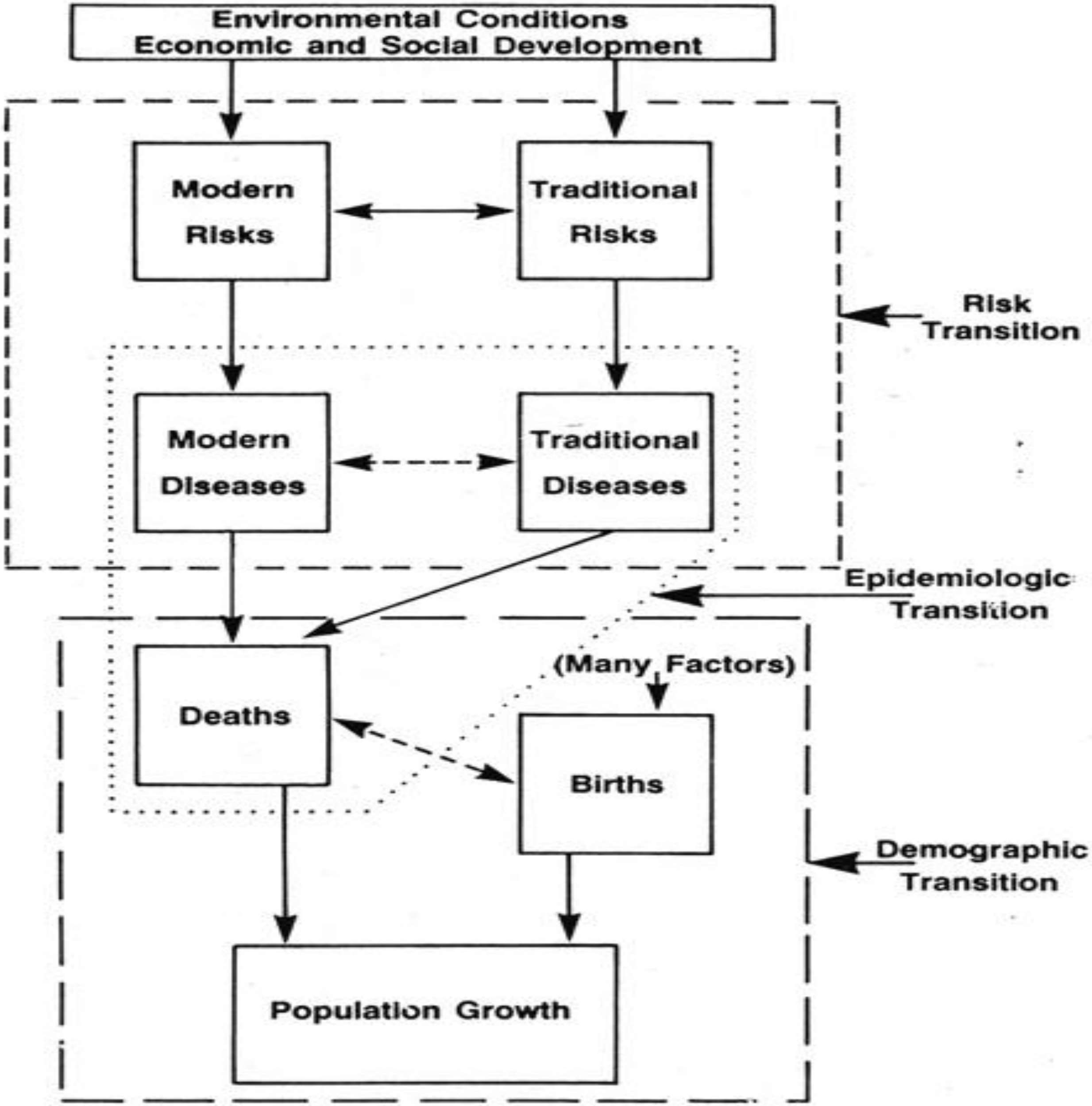
Echolocation

- Capability is found mostly in microchiropteran bats
- Signals are produced by the larynx, powered by the muscles of the abdominal wall, and emitted through the mouth or nostrils
- Droplets or small-particle aerosols of oropharyngeal fluids, mucus, and saliva could accompany sound generation
- Mucus from naturally infected Mexican free-tailed bats containing rabies virus was isolated

Risk, Safety and Personal Protective Equipment

Ian H Mendenhall

THE HEALTH TRANSITION



Risk

Exposure to danger

Potential for something bad to happen

What are some risks you face?

Hazard

An agent with the potential to cause morbidity or mortality

Situation with potential for something bad to happen

Risk versus Hazard

Hazard – something with potential to cause harm

Risk – likelihood of something happens and the relative consequences when the hazard occurs

Severity versus Likelihood

For every action taken, we must assess the likelihood of occurrence compared to the severity of that outcome

- What is the likelihood of hurting your ankle walking to a field site and what is the severity of that injury?

- What is the likelihood of the autoclave exploding and what would the severity of that injury be?

Risk Management

		Consequences		
		Minor 3	Moderate 2	Major 1
Likelihood	Probable A			X
	Possible B			
	Improbable C			

Key	Green Low Risk	Yellow Medium Risk	Red High Risk

http://exploringpossibilityspace.blogspot.sg/

Risk Analysis = adding science to policy and/or decision making.

Applying science to policy addressing real problems

Three general components of assessing risk

- Identify Hazard(s) = what are we concerned about?
- Assess Vulnerability = of whom?
- Assess Impact = likelihood and magnitude

The potential that a given threat will exploit vulnerabilities to have an impact, usually negative in this sense

Transparency = the process must be inclusive and completely transparent in terms of process and assumptions

Evaluate every task

For a new protocol, we need to evaluate every task from beginning to end in an effort to minimize risk

- Follow a flow chart to idenitfy areas of high risk and how we can mitigate this risk

For example: We are establishing primary bat cell lines from multiple tissue types. What are the risks?

Risk Assessment

What is the risk (frame the question)?

What is the hazard?

What are the risk pathways?

Where are knowledge gaps?

Can we collect data to fill these gaps

Using this information, can we assess the risk?

Risk of Nipah virus into Singapore

	Define	Steps to Occur	Data
Release	Likelihood of occurrence	Bat is infected. Bat visits Singapore.	Are bats likely to visit? Are local bats susceptible?
Exposure	Likelihood of human exposure	Infected bat and human share space-time.	Where would bats visit? Do humans frequent?
Consequence	Consequences	Human is infected. Potential human-human transmission	Type of exposure (fomite, direct)

Data

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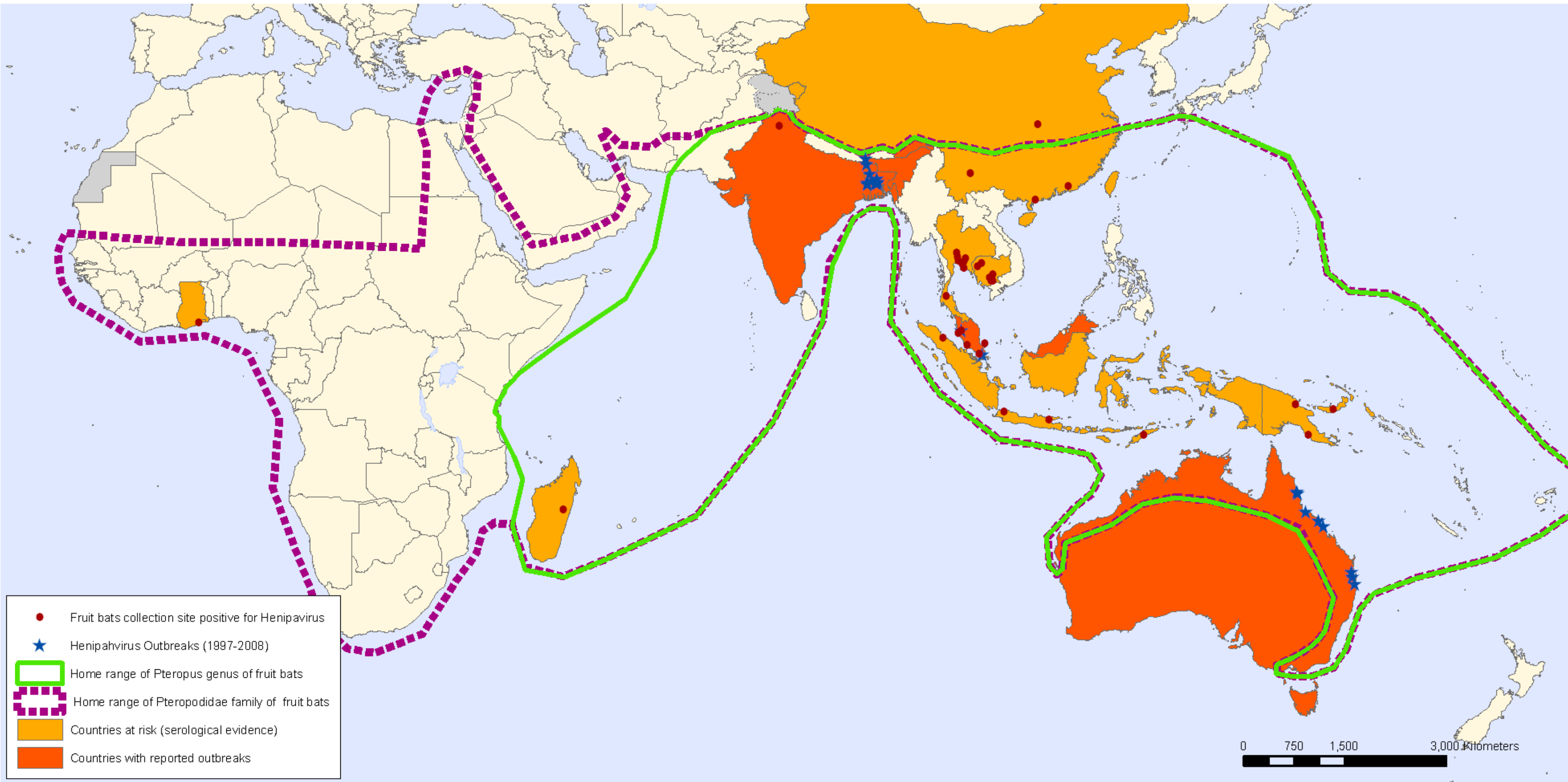
Geographic distribution of Henipavirus outbreaks and fruit bats of Pteropodidae Family



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: Global Alert and Response Department
World Health Organization
Map Production: Public Health Information
and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

Risk Management & Risk Communication

How do you safely insure your staff (and yourself) are protected?

Can you evaluate alternative approaches without compromising safety?

Do you have reliable people to ask their opinions?

(OIE Code, 2011)

Risk Assessment of Nipah in Singapore

Is the risk:

Negligible, Low, Medium, High, Very High?

If the risk is low, we can allocate energy and resources to other areas.

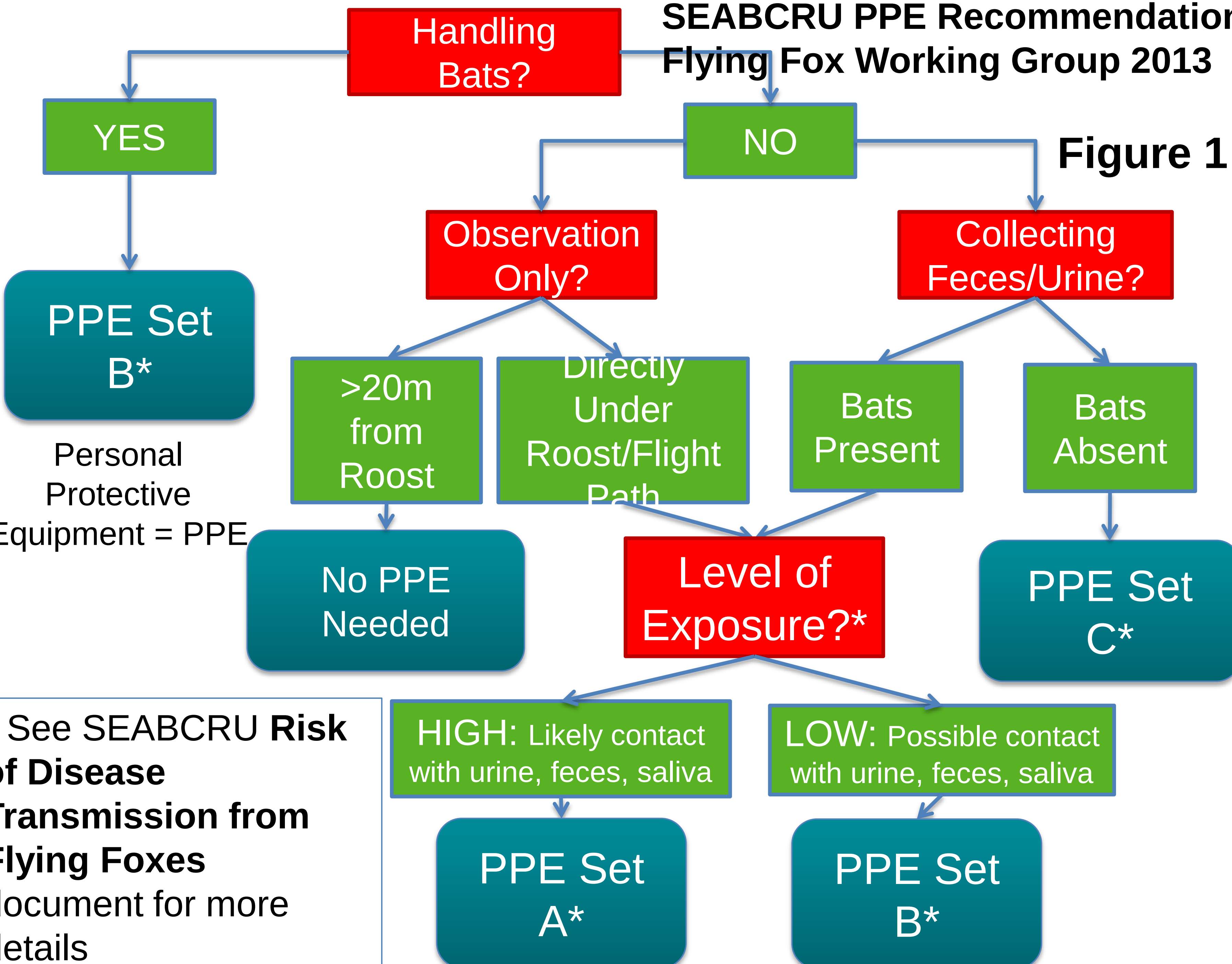
Personal Safety & Equipment

Is there too much personal protection?

Should we always wear the most protective outfits?

SEABCRU PPE Recommendations Flying Fox Working Group 2013

Figure 1

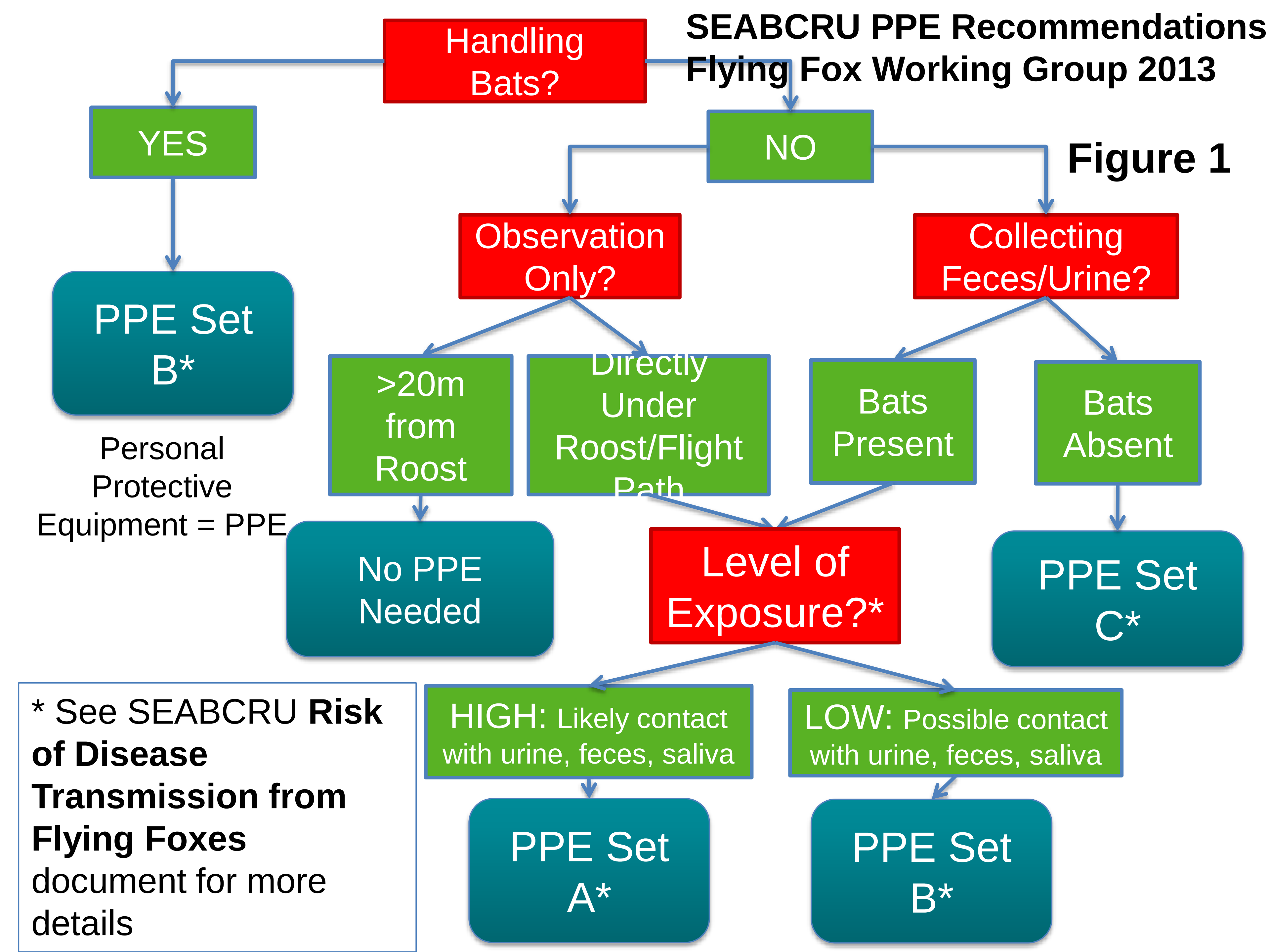


Personal Protective Equipment = PPE

* See SEABCRU **Risk of Disease Transmission from Flying Foxes** document for more details

PPE Set A (High exposure to bat fluids likely)

- Tyvek suit (or disposable rain coat over dedicated clothing)
- Eye protection (goggles or safety glasses)
- Mask (N95 or P100 respirator or comparable)
- Gloves (Thick nitrile gloves for handling bats, otherwise latex okay for roost samples)
- Covered shoes (that can be disinfected)
- Boot covers (optional depending on activity)


SEABCRU PPE Recommendations
Flying Fox Working Group 2013
Figure 1
Handling Bats?
YES
NO
PPE Set B*
Personal Protective Equipment = PPE
Observation Only?
Collecting Feces/Urine?
>20m from Roost
Directly Under Roost/Flight Path
Bats Present
Bats Absent
No PPE Needed
Level of Exposure?*
PPE Set C*
HIGH: Likely contact with urine, feces, saliva
LOW: Possible contact with urine, feces, saliva
PPE Set A*
PPE Set B*
* See SEABCRU Risk of Disease Transmission from Flying Foxes document for more details

PPE Set B (Handling bats or probable fluid exposure)

- Dedicated clothing (= long sleeve shirt, long pants that are removed after finishing field work and not worn home)
- Eye protection (goggles or safety glasses)
- Mask (N95 or P100 respirator or comparable)
- Gloves (Thick nitrile gloves for handling bats, otherwise latex okay for roost samples)
- Covered shoes (that can be disinfected)

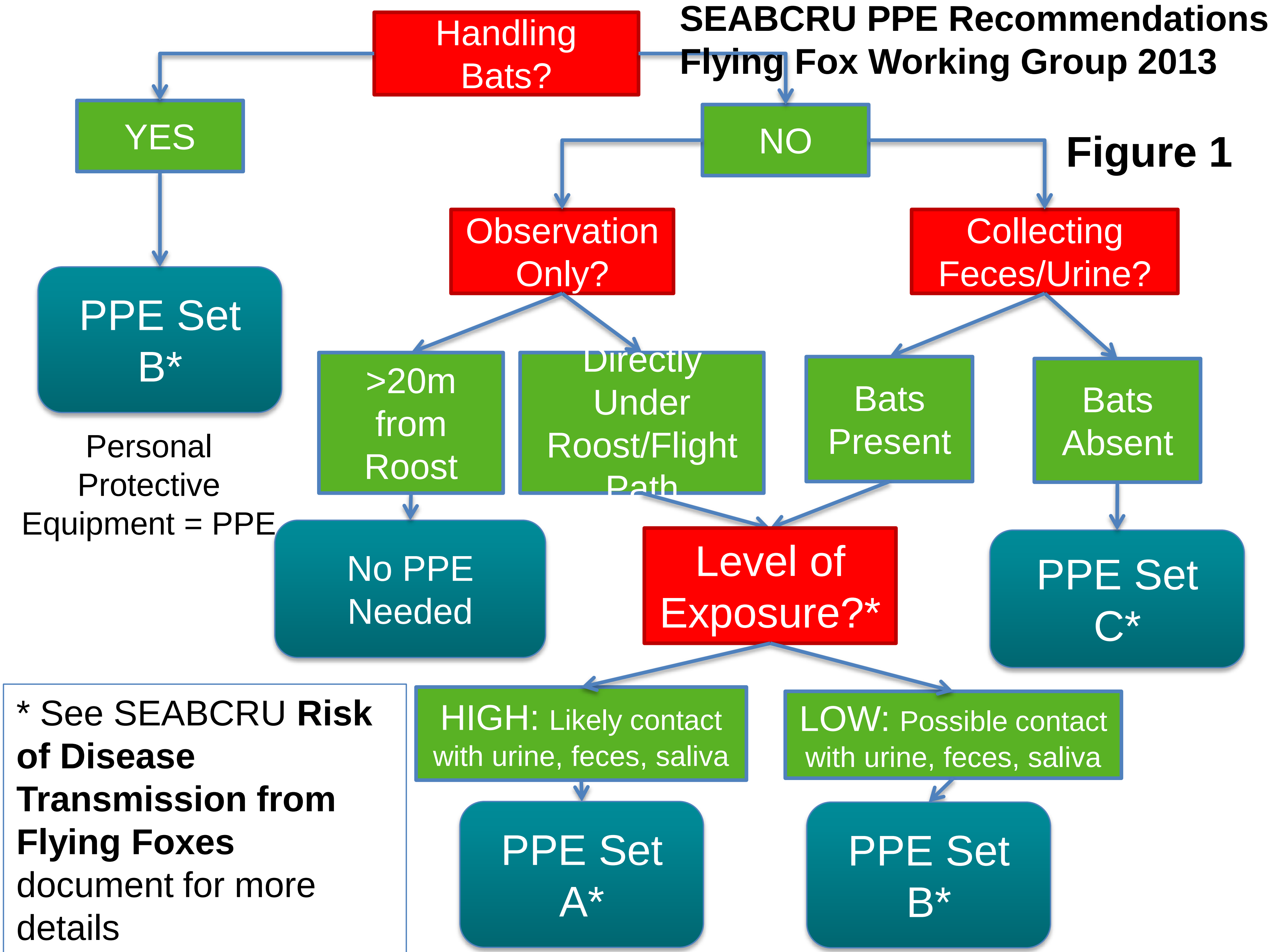

SEABCRU PPE Recommendations
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Figure 1
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YES
NO
PPE Set B*
Personal Protective Equipment = PPE
Observation Only?
Collecting Feces/Urine?
>20m from Roost
Directly Under Roost/Flight Path
Bats Present
Bats Absent
No PPE Needed
Level of Exposure?*
PPE Set C*
HIGH: Likely contact with urine, feces, saliva
LOW: Possible contact with urine, feces, saliva
PPE Set A*
PPE Set B*
* See SEABCRU **Risk of Disease Transmission from Flying Foxes** document for more details

PPE Set C (Lower exposure to bat fluids)

- Dedicated clothing (= long sleeve shirt, long pants that are removed after finishing field work and not worn home)
- Mask (N95 or P100 respirator or comparable)
- Gloves (latex okay)
- Covered shoes (that can be disinfected)

Mitigation of Likelihood

Don’t need self-contained respirator

Gloves

N95 mask

Long sleeves

Head covering (shower cap/hankerchief)

Eye protection

Mitigation of Severity

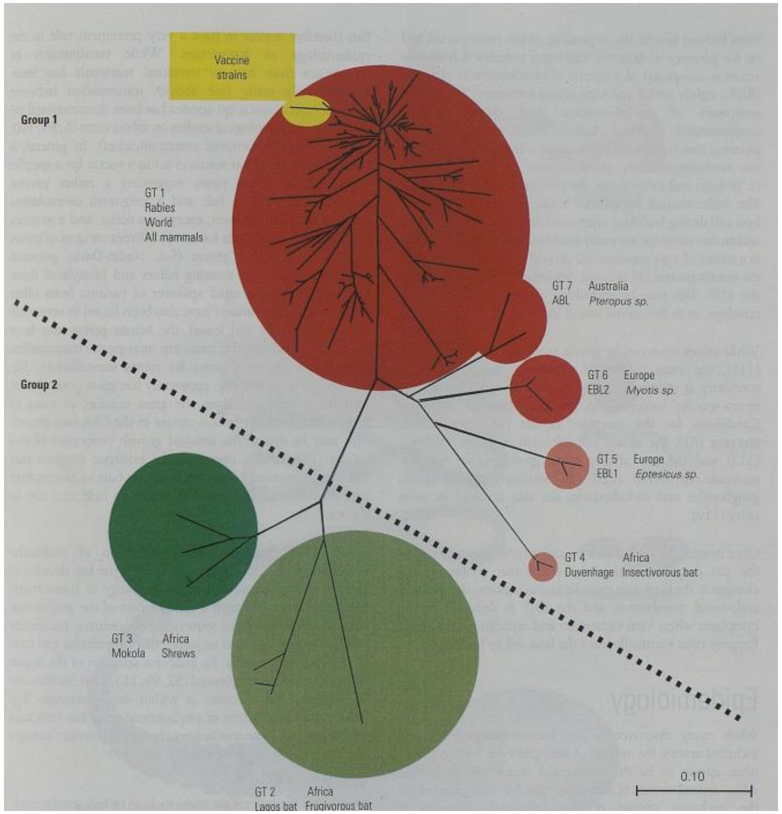
Vaccine. 2012 Dec 14;30(52):7447-54. doi: 10.1016/j.vaccine.2012.10.015. Epub 2012 Oct 19.

Rabies virus vaccines: is there a need for a pan-lyssavirus vaccine?

Evans JS[1], Horton DL, Easton AJ, Fooks AR, Banyard AC.

Lack of vaccinations for majority of bat viruses

Prevention is better



Reticence

Reluctance of ecology workers to employ these PPE

Why?

- Extra expenses
- It's hot
- We've been doing it this way for years
- Assumed low risk

Risk Analysis – What to consider?

- Ebola or Nipah outbreak

- Routine surveillance
  - Level of contact
  - What species are we working with? Are they suspected reservoirs?
  - Simple background data study?
  - What is your plan when you find a pathogenic agent?

Risk Analysis

Assess risk with sampling bats

- Human outcomes of infection
- Primary reservoir(s)?
- Avenue of infection?
- How would you approach each outbreak?
- How do you deal with diagnostic samples?

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Thank You

Partner in Academic Medicine

SingHealth

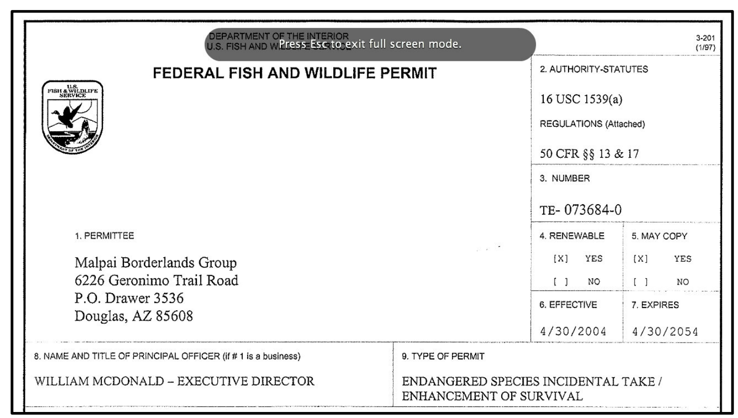
www.duke-nus.edu.sg

Capture and handling of bats

“A bat in a hand is worth two in the cave”

Permits

- Bats – protected fauna in some countries
- Illegal to catch or keep them in captivity
- Pest species may require a permit for capture
- Permits are issued by state national park, wildlife/ forest authority or environmental protection agency
- Competency of scientists/handlers may be assessed, and vaccination needed


DEPARTMENT OF THE INTERIOR
U.S. FISH AND WI

Press Esc to exit full screen mode.

3-201 (1/97)

FEDERAL FISH AND WILDLIFE PERMIT

U.S. FISH & WILDLIFE SERVICE

2. AUTHORITY-STATUTES	16 USC 1539(a)
REGULATIONS (Attached)	50 CFR §§ 13 & 17
3. NUMBER	TE- 073684-0
4. RENEWABLE	[X] YES [ ] NO
5. MAY COPY	[X] YES [ ] NO
6. EFFECTIVE	4/30/2004
7. EXPIRES	4/30/2054

1. PERMITTEE

Malpai Borderlands Group
6226 Geronimo Trail Road
P.O. Drawer 3536
Douglas, AZ 85608

8. NAME AND TITLE OF PRINCIPAL OFFICER (if # 1 is a business)

WILLIAM MCDONALD – EXECUTIVE DIRECTOR

9. TYPE OF PERMIT

ENDANGERED SPECIES INCIDENTAL TAKE / ENHANCEMENT OF SURVIVAL

- Permits / referees needed for net purchase

Personal safety and protective equipment

- Field workers may be bitten or scratched
- Other animals such as owls, squirrels and insects
- Need to be safely removed
- Advisable to carry a range of PPE for safe handling of animals
- Water safety
- Details will be covered later

Choice of capture methods/devices

- Taxa of interest
- Number of animals expected at a site
- Habitat type
- Availability of field assistants

Capture success is affected by **weather, local topography, moonlight conditions, and habitat**.

How do these variables affect capture success in a given study? Are there potential biases?

Equipment and techniques for capture

- **Hand nets**
  - Hand net / butterfly net attached to a telescopic pole with a large diameter hoop
  - Useful for confined spaces such as caves, mines and buildings

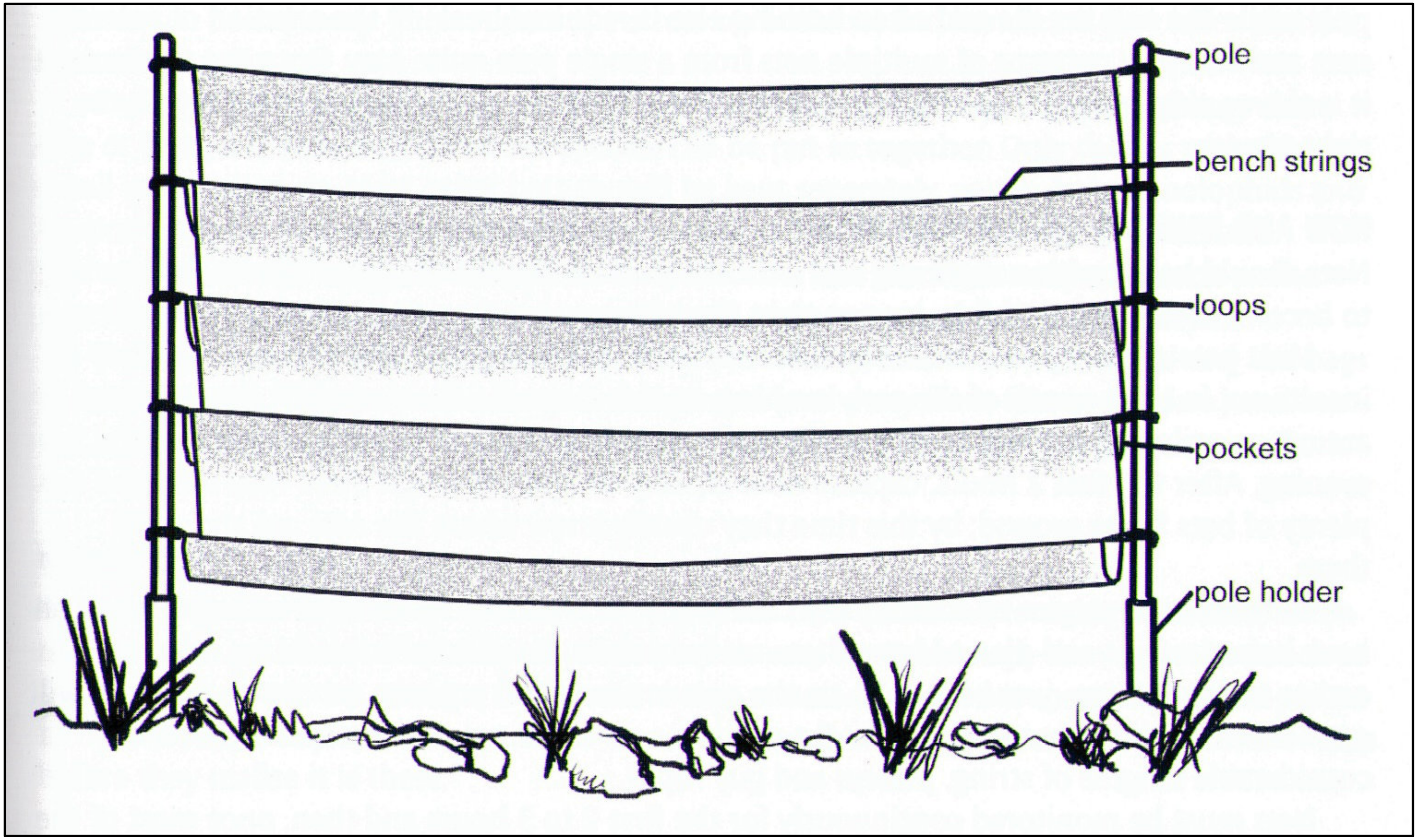
  - May be used in conjunction with a physical lure
  - Caution in using hand nets – not to strike too hard

Equipment and techniques for capture

- **Mist nets**
  - Common, convenient and inexpensive
  - Made of nylon or terylene (polyester monofilament)
  - Terylene: softer, more durable, highly effective
  - Nylon: stiffer, cheaper, great for rough environments like caves and mines
- **Mist net poles**
  - Made of rough bush poles, bamboo, aluminum

Equipment and techniques for capture

Mist nets



Equipment and techniques for capture

- **Mist nets** – specifications

Mesh size – distance between the 2 diagonal corners in the mesh of a stretched net

Denier – Number of grams of nylon in 9,000 m of fibre and is reflected in the "weight" or thickness, of the fibre

Ply – Number of fibres that make up a braided strand

e.g. 50-denier, 2-ply nylon = 2 strands of nylon fibre (9,000 m of which weighs 50 g) braided together to form the thread

Small bats – mesh size 36-38mm / 30, 50, 70 denier / 2ply

Flying foxes – mesh size 100 mm, > 2 m in height, 4 pockets

Heavier denier = more durable, but easily detected visually and acoustically

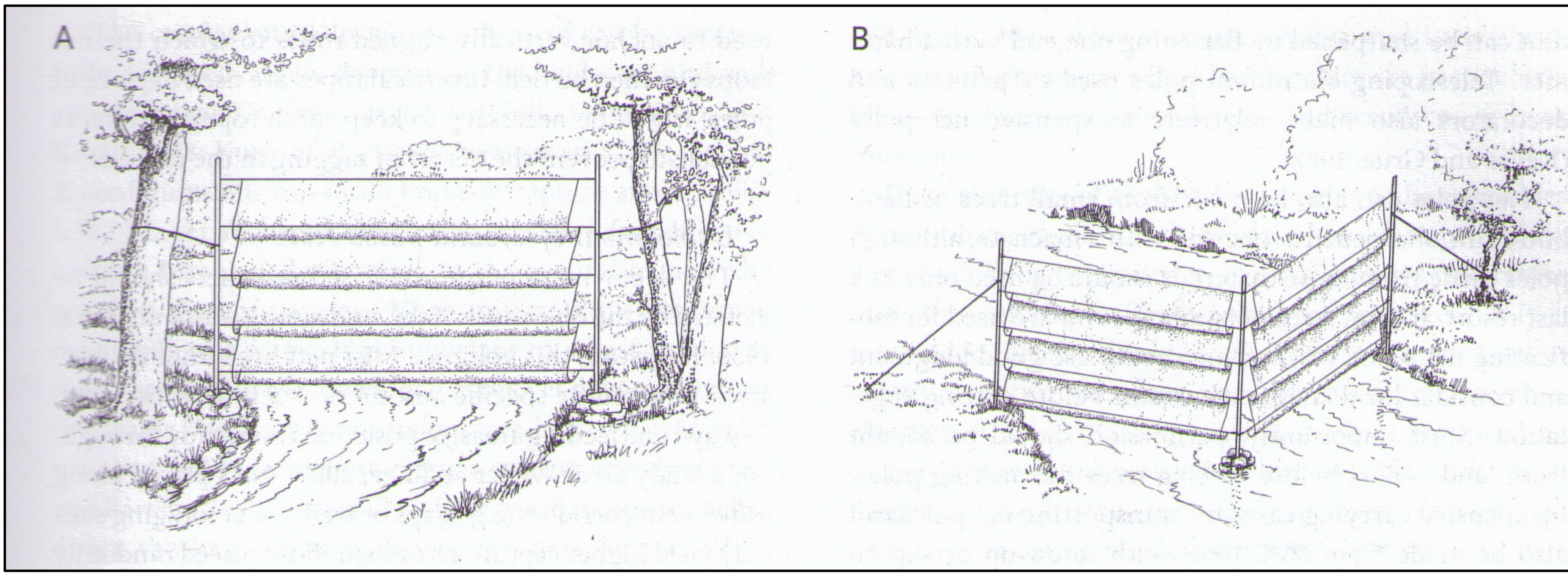
Equipment and techniques for capture

Mist nets – specifications

- Lengths 3, 6, 9, 12 and 18 m and range in height from 2.3 to 2.6 m
- Macro nets – 30 m long and 6 m high, achieved by stacking or joining
- Most versatile mist nets – 6 and 9 m; cluttered habitats, across streams and trails, and around foliage roosts
- Long nets – open country, large forest gaps, ponds and rivers
- Nets should be checked every 15 minutes

Equipment and techniques for capture

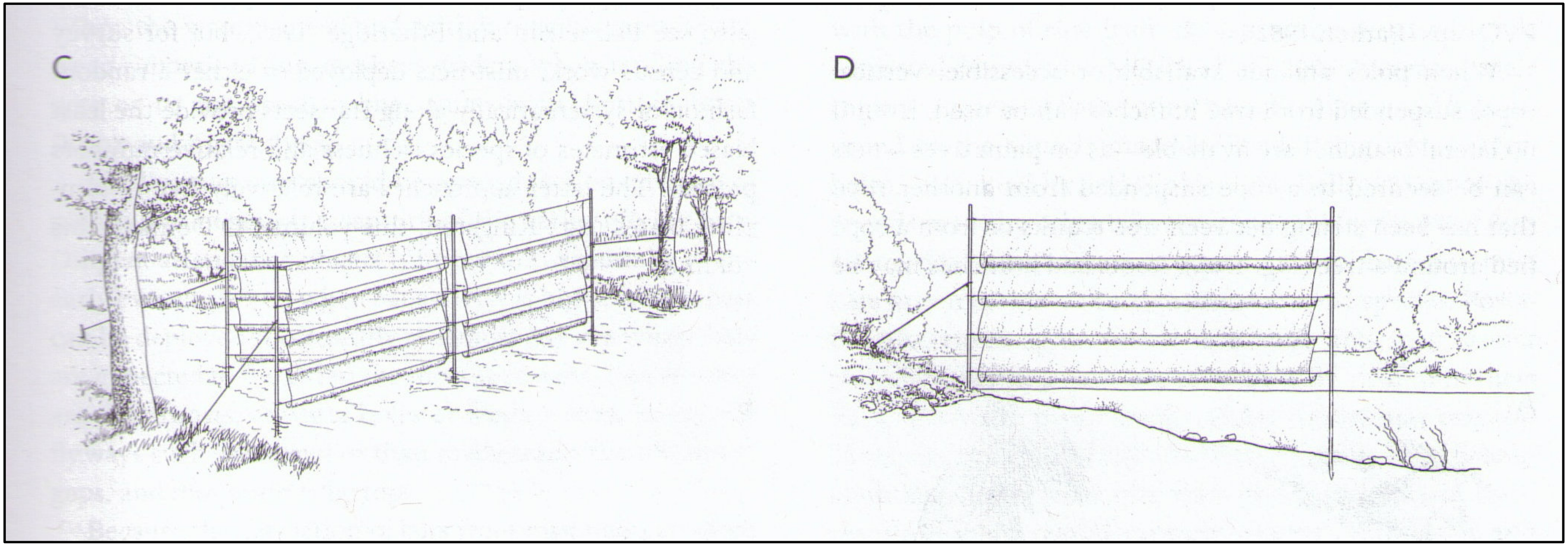
Mist nets – configuration and field deployment



A: single net over a stream, with poles tied to trees
B: two nets in a V-configuration set over a stream

Equipment and techniques for capture

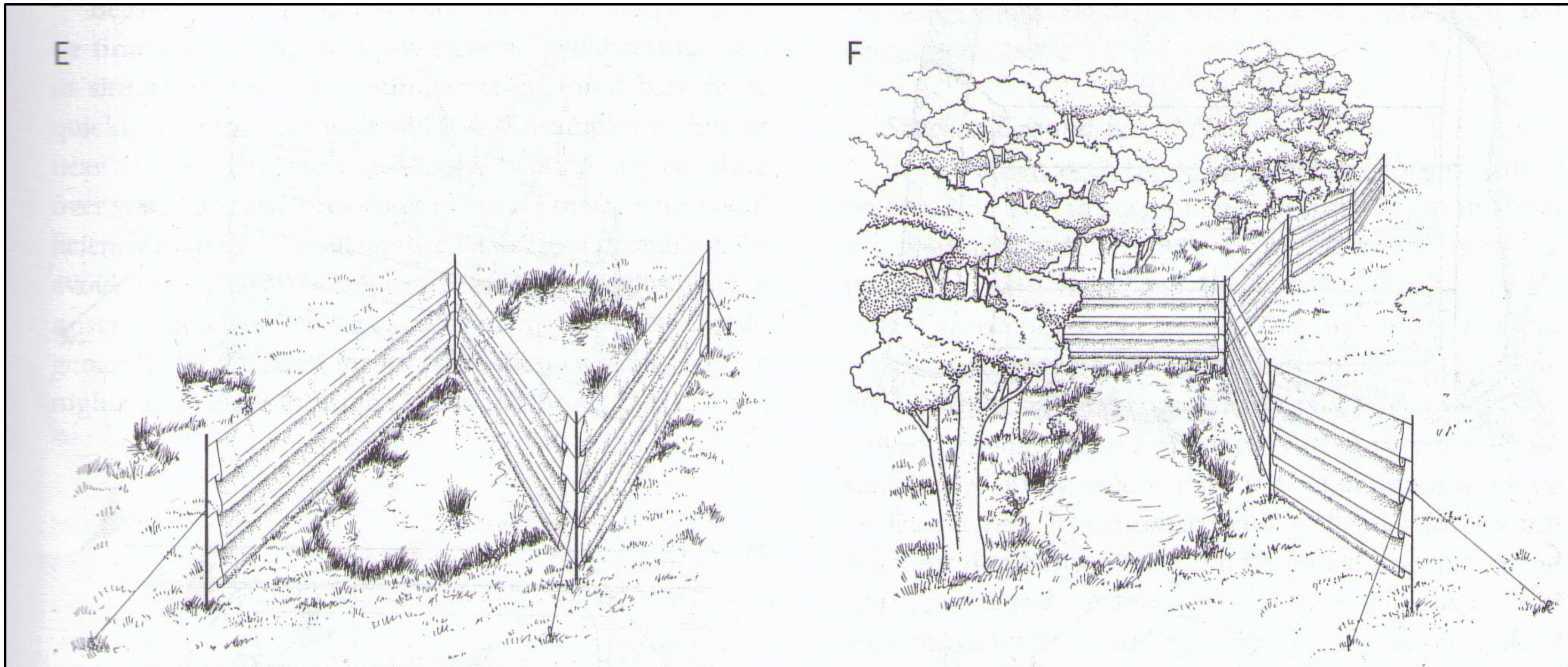
Mist nets – configuration and field deployment



C: T-net configuration over a small pond
D: single net set along the edge of a stream/pond, with long pole driven into substrate

Equipment and techniques for capture

Mist nets – configuration and field deployment

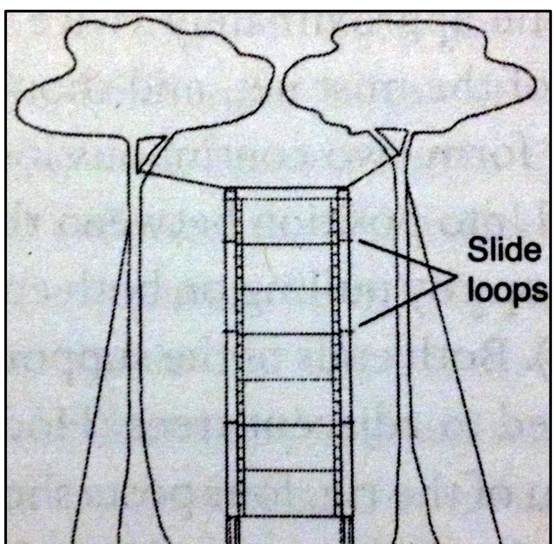


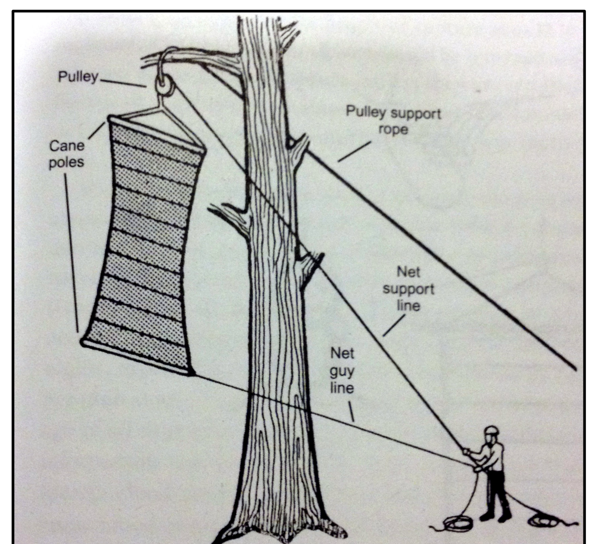
E: Z-net configuration over a pond
F: Y-net configuration in a diverse landscape

Equipment and techniques for capture

High nets / canopy nets

- Used in forest sub-canopies to evaluate vertical stratification of bat assemblages
- Also catch high-flying species
- High mist nets, canopy net (horizontal and vertical)





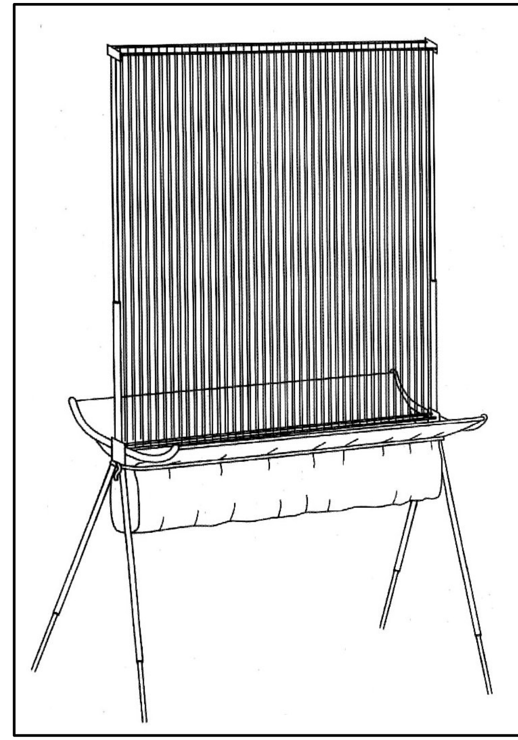
Equipment and techniques for capture

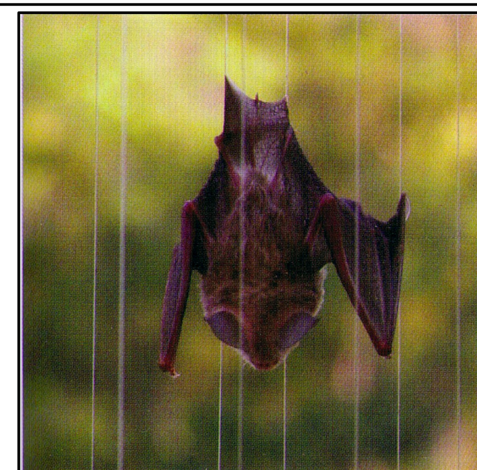
High nets – 24 ft poles (7.3 m) with mist net strung across (see http://www.batnets.com/ to build/acquire poles.

Equipment and techniques for capture

- **Harp traps**
  - one or two supporting rectangular frames
  - monofilament fishing lines strung vertically, spaced 2-3 cm apart
  - 2,3 or 4 banks
  - catching bag at the bottom of the trap

  How it works:
  - Vertical lines hard for bats to detect
  - Hit the lines and they fall guided by the lines into the bag below



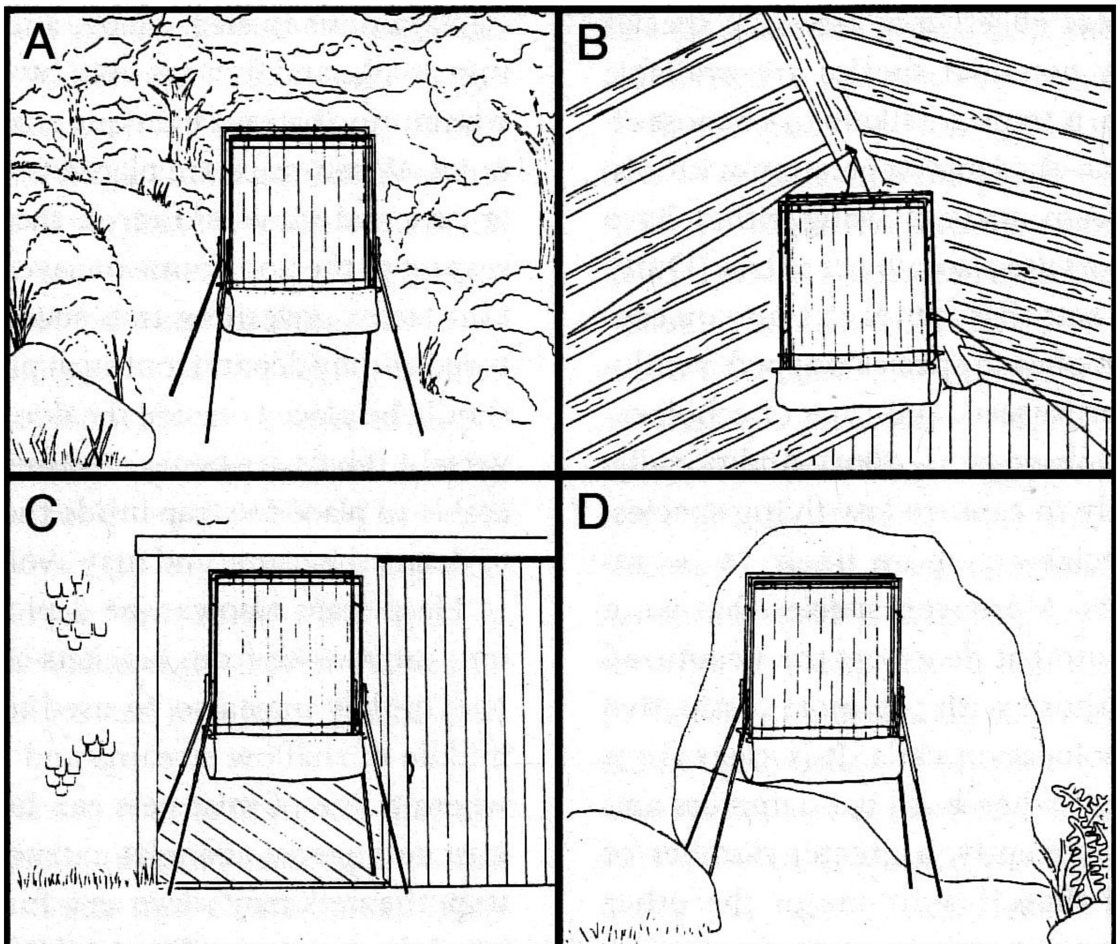


Equipment and techniques for capture

- **Harp traps**
  - can be used on uneven terrain – adjustable legs
  - set during the day and left unattended at night
  - useful for cave entrances
  - check the traps several times a night and remove captured bats
  - could be used in combination with mist nets

Equipment and techniques for capture

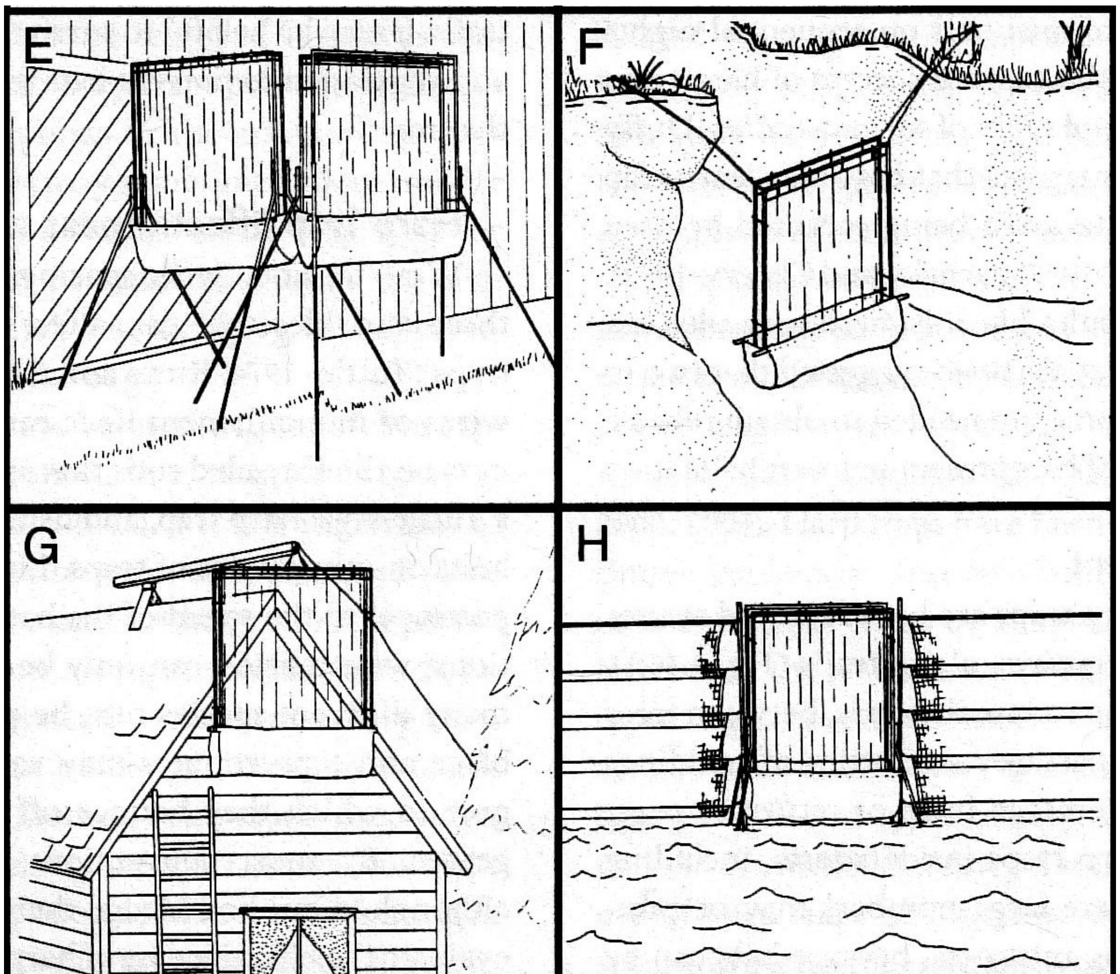
- **Harp traps -** placement



A: forest trail
B: suspended beneath the ridge pole in a barn
C: doorway
D: cave entrance

Equipment and techniques for capture

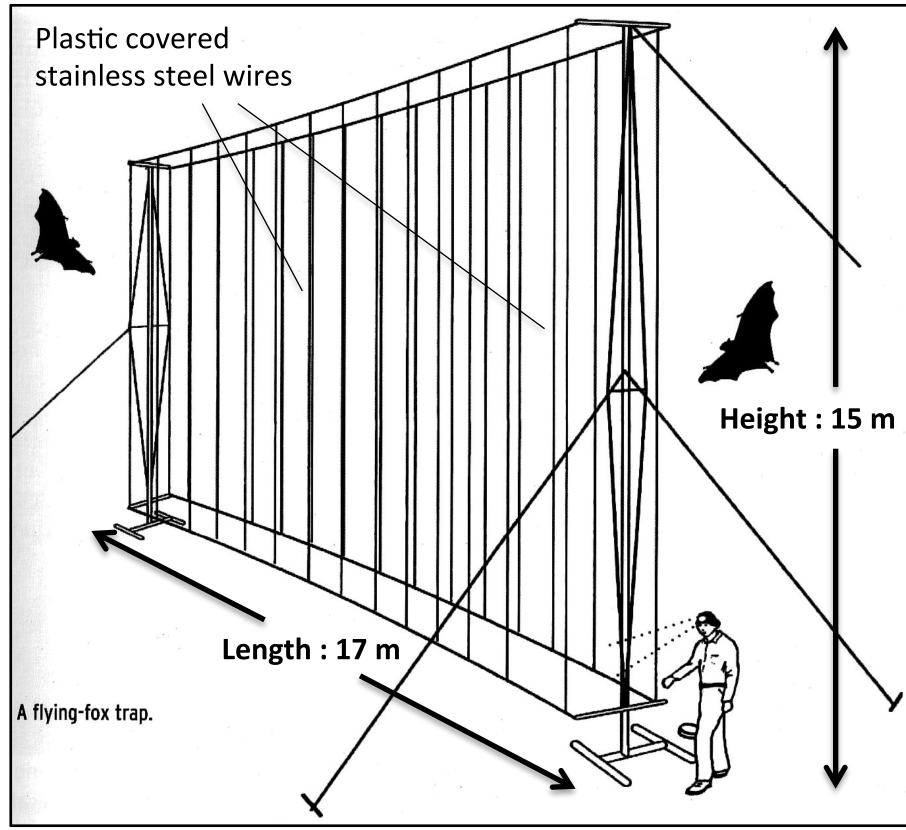
- **Harp traps -** placement



E: L-configuration in front of a closed door (opposite a crevice)
F: suspended in a canyon
G: suspended outside a building (opposite a crevice)
D: shallow pond and used with mist-nets

Equipment and techniques for capture

- **Mega harp trap**
  - invented by Tidemann and Loughland (1993)
  - catching area ~ 220 m^2
  - cables strung between two yacht masts
  - set up takes three persons
  - continuously monitored
  - no catching bag at the bottom, bats are caught as they slide to the ground



Equipment and techniques for capture

Enhancing capture rates (of insectivorous bats)

- Use of acoustic lures with mist nets / harp traps
  - Play back the call of a bat to attract other bats

- Use of UV light to attract insects
  - Concentrate bat activity where you are sampling

References

- Kunz, T.H., et al. (2009) Methods of capturing and handling bats. In: Ecological and behavioral methods for the study of bats. Eds. Kunz, T.H. and Parsons, S. pgs. 3-35.

Samples, Storage, and Data

Ian H Mendenhall

Identifying goals

Surveillance for particular viruses, bacteria, protozoa, helminth or fungi of public health interest

Monitor and understand long term trends

Safely handle samples

- Issue with diagnostic samples

SWOT

Strengths

Weaknesses

Opportunities

Threats

Gold Standard

Live virus

RNA viruses

DNA viruses

DNA of host

RNA of host

Bacteria

Protozoans

Helminths

How to screen?

Species as known reservoirs

Species confirmed as infected or seropositive

Species of unknown status

Whatever I can catch

Tissues that have known tropisms

Entire organisms (ground tissues)

Potential aberrant infections

Isolation, PCR, ELISA, Microneuts?

Pathogen Matrix

		DNA		RNA Viruses													Bacteria									Protozoa			Helm
Animal	Sample	Adenovirus	Herpesvirus	Arenavirus	Astrovirus	Bunyavirus	Coronavirus	Filovirus	Flavivirus	Hantavirus	Orthomyxovirus	Paramyxovirus	Picornavirus	Reovirus	Rhabdovirus	Togavirus	Rickettsias	Ehrlichia	Neorickettsia	Bartonella	Leptospirosis	Borrelia	Plague	Mycoplasma	Anaplasma	Plasmodium	Babesia	Trypanosoma	Nematodes
Bat	Blood (RBC)																x	x	x	x		x			x	x	x	x	x
	Sera							x	x							x													
	Oropharyngeal swab/saliva	x	x								x																		
	Anal swab/feces	x			x		x	x			x																		
	Ectoparasites					x			x		x					x	x			x		x					x		
	Urine	x	x									x		x										x					
Bird	Blood (RBC)																					x			x	x			x
	Sera					x			x							x													
	Oropharyngeal swab/saliva										x																		
	Cloacal swab/feces	x			x		x				x																		
	Ectoparasites					x					x																		
Small Mammal	Blood (RBC)																			x		x			x	x	x	x	x
	Sera			x												x							x						
	Oropharyngeal swab/saliva			x							x																		
	Anal swab/feces				x						x																		
	Ectoparasites									x							x						x				x		
	Urine			x						x							x												
Primate	Feces																									x			
Mosquitoes	Homogenate					x			x							x										x			x

Samples

All collected in interest of not harming bat, but collecting valuable sample

- Time/money investment requires return
- Use polyester, Dacron, or rayon swabs (no cotton, no wood)

Oropharyngeal swabs – collect sufficient material

Anal swabs or feces – care not to cause damage

Blood & smear – properly dilute (1:10)

Media

Viruses – virus transport media

- Anti-bacterial, anti-fungal solution
- Store at -20

Bacteria – media or PBS+glycerol

Macroparasites – ethanol (90-95% for DNA work)

Ectoparasites – depends on final goal (mounting vs DNA vs parasite detection

RNALater – preservation of nucleic acids

Environmental & Ecological Samples

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Collection of urine or feces under roost

Define:

- Habitat type
- Canopy cover
- Ground cover
- Vegetation assemblage
- Wind, temperature, precipitation, moon phase during collections

These will tell you expected presence-absence

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Transport – WHO guidelines

Specimen	Transport media	Storage condition		Purpose/ Lab investigation
		Transport	Pending test	
Throat swab	VTM	2-8 ^{0}C	-20 to -80 ^{0}C	Isolation
NPA/ swab	VTM	2-8 ^{0}C	-20 to -80 ^{0}C	Isolation
CSF	No	2-8 ^{0}C	-20 to -80 ^{0}C	Isolation, serology
Stool	No	2-8 ^{0}C	-20 to -80 ^{0}C	Isolation
Urine	No	2-8 ^{0}C	-20 to -80 ^{0}C	Isolation
Serum/ Clotted blood	No	2-8 ^{0}C	-20 ^{0}C 2-8 ^{0}C	Isolation, serology
Whole blood	No	2-8 ^{0}C	2-8 ^{0}C	Isolation, serology

Cold Chain

What kind of cold chain do you have?

How can I treat my sample?

Categorize freeze-thaw cycles

Luxury of -80C and liquid nitrogen tank

Do you have space?

Labeling

Background in databases?

Have unique identifiers for each sample and animal

- How to handle multiple trips on same day?
- Data entry?

Temporary vs Permanent labels

- Duplicates?

Centers of Excellence for Influenza Research or PREDICT standards

- Standardize data collection so we can make global comparisons

Example Goal

How can we make a project One Health?

Nipah virus or MERS surveillance

- Collecting particular samples
- Are the additional low hanging fruits, pending time and labor?

Opportunities

- Do we have time/human resources to take other samples?

- Can we collect for the future?
  - Value in sample banking – what will future technology permit?
  - PanBio sequencing

- Can we entice local universities or overseas partners?

- Academic success driven by funding and publications

Opportunities

Collect samples specifically for target parasite

- Oral and anal swabs, blood, sera, ectoparasites
- Morphometrics, gender, age
- Harvest animal (duplicate for RNA analysis)
- Environmental data

Future projects

- Blood borne parasites
- Ectoparasite diversity
- Population genetics of host
- Diet – prevalence and trends of insects, flowers, fruit
- Reproductive/partuition peaks

Big Question

Where do we sample?

How often do we sample?

Tiny spatial-temporal windows

What samples do we collect?

Are the resources available to drive future investigations?

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Thank You

Partner in Academic Medicine

SingHealth

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