

Voucher specimen preparation: bats

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Part of the Darwin Initiative Project: *Taxonomic initiative for Southeast Asian bat studies (Vietnam, Thailand, Cambodia and Lao PDR)*



Data recording whilst in the field

Field data

A requirement for taxonomic studies is that essential data are recorded for all voucher specimens sacrificed in the field. These data should be recorded in a strong field notebook (preferably) or on data sheets (these must be bound into a book on return from the field and stored as archive material). The following information should be noted:

- Unique identity code¹, which is recorded both in the notebook and on a label², which is attached to the right foot of the specimen. PB100505.1 0
- Date of collection
- Locality data – including nearest point of reference (eg village, hill..), district, department, province, country and geographical co-ordinates in degrees and minutes eg., 'N 'E Unique identity code on a label made of durable card or dymo tape – a thin string attaches it to the foot of the specimen
- Sex of specimen
- Provisional species identification
- Basic external measurements³ – by preference these should be taken from fresh specimens but can be taken later from specimens stored in alcohol

Spec. No.	Date		Locality				Species	Sex
	HB	Tail	FA	HF	E	Mass		
	10.06.2005		Ai-kading stream, Bala Forest, Hala-Bala Wildlife Sanctuary, Wang District, Narathiwat Province, Thailand, 05°48.15'N, 101°49.25'E					
PB100505.1	40.0	37.1	38.7	9.8	15.1	7.0	<i>Pipistrellus stenopterus</i>	♂
PB100505.2	40.5	35.7	39.6	8.5	13.2	7.1	" "	♀
PB100505.3	50.0	26.0	48.1	10.1	20.0	6.2	<i>Rhinolophus acuminatus</i>	♂
	12.07.2005		Saddan-Sin Cave, 10 km north of Mawlamyine, Mon State, Myanmar, 16°44.01'N, 97°43.23'E					
PB120605.1	44.3	22.9	41.3	7.4	16.9	6.8	<i>Rhinolophus malayanus</i>	♂

Part of a page from a [hypothetical] field notebook

Unique Identity code¹

- The unique identity code should be prefixed with a letter (letters) – each expedition or field study should have a different letter prefix which may or may not correspond to some aspect of the expedition (ie SH or SS for a field study in Shan State). Each voucher specimen then receives a different number after the letter eg SH1, SH2, SH3.....
- Alternatively the field number used may relate to the country/ or region within a country and the date of collection, such that MY100505.1 is the first specimen collected by the field team in Myanmar on 10 May, 2005. Or it may refer to the collector and the date of collection, eg. the first specimen collected by Paul Bates on 10 June, 2005 would be PB100505.1 .

Field label²

- Should be of durable card or plastic dymo tape
- Have a thread for attaching to the bat's right hind foot
- Have a unique identity code embossed on the dymo tape or written in permanent (Indian) ink pen/ or pencil (never biro – it fades or spoils in alcohol very quickly)

External measurements and weight³

- Head and body length: from the tip of the snout to the base of the tail, dorsally
- Tail length: from the tip of the tail to its base adjacent to the anus
- Forearm length: from the extremity of the elbow to the extremity of the carpus with the wings folded
- (Hind) foot length: from the extremity of the heel behind the *os calcis* to the extremity of the longest digit, not including the hairs or claws
- Ear length: from the lower border of the external auditory meatus to the tip of the pinna

For an illustration and definition of these and many other external and cranial measurements, see attached pages xv-xvi taken from Bates and Harrison (1997).

Bates, P.J.J. and D.L. Harrison. 1997. Bats of the Indian Subcontinent. Harrison Zoological Museum Publications. 258 pp.

Notes:

Definitions of Measurements

External Measurements

HB: head and body length - from the tip of the snout to the base of the tail, dorsally (Fig. i).

T: tail length - from the tip of the tail to its base adjacent to the body (Fig. i).

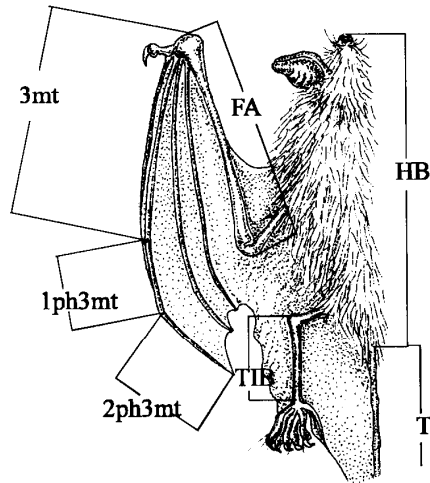


Fig. i. Left wing and body of *Myotis sicarius*.

HF: foot - from the extremity of the heel behind the os calcis to the extremity of the longest digit, not including the hairs or claws.

TIB: length of tibia - from the knee joint to the ankle (Fig. i)

FA: forearm - from the extremity of the elbow to the extremity of the carpus with the wings folded (Fig. i)

WSP: wing span - maximum spread, from tip to tip, with the wings fully extended.

Thumb: length of thumb (first digit), including metacarpal, phalanx and claw.

3mt (MET): third metacarpal - from the extremity of the carpus to the distal extremity of the metacarpal (Fig. i).

4 mt (MET); 5mt (MET): as above but for the fourth and fifth metacarpals respectively (Fig. i).

1ph3mt: first phalanx of the third metacarpal - taken from the proximal to the distal extremity of the phalanx (Fig. i).

2ph3mt: second phalanx of the third metacarpal - taken from the proximal to the distal extremity of the phalanx (Fig. i).

1ph4mt/ 2ph4mt: as above but for the fourth metacarpal.

E: ear - from the lower border of the external auditory meatus to the tip of the pinna, not including any tuft of hair (Fig. ii).

Cranial and dental measurements

GTL: greatest length of skull: the greatest antero-posterior diameter of the skull, taken from the most projecting point at each extremity, regardless of what structure forms these points (Fig. iv, v).

CBL: condylo-basal length - from an exoccipital condyle to the alveolus of the anterior incisor (Fig. iv).

CCL: condylo-canine length - from the exoccipital condyle to the anterior alveolus of the canine (Fig. iv).

ZB: zygomatic breadth - the greatest width of the skull across the zygomatic arches, regardless of where this point is situated on the arches (Fig. iii).

BB: breadth of braincase - greatest width of the braincase at the posterior roots of the zygomatic arches (Fig. iii).

IC: interorbital constriction - the narrowest width across the interorbital region.

PC: postorbital constriction - the narrowest width across the constriction posterior to the orbits (Fig. iii).

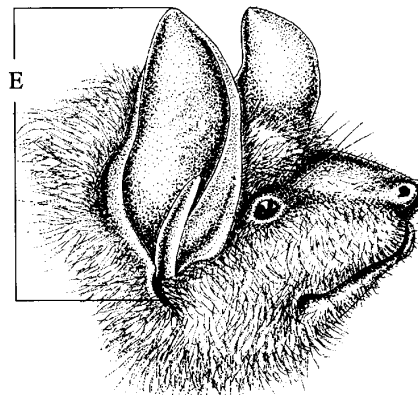


Fig. ii. Right ear and face of *Myotis horsfieldii*.

M: mandible length - from the most posterior part of the condyle to the most anterior part of the mandible, including the lower incisors (Fig. v).

RW: rostral width - taken across the front of the orbits at their most anterior point (Vespertilionids only).

C-Mⁿ: maxillary toothrow - from the front of the upper canine to the back of the crown of the last upper molar (Fig. v).

$C-M_n$: mandibular tooththrow - from the front of the lower canine to the back of the crown of the last lower molar (Fig. v).

C^1-C^1 : anterior palatal width - taken across the outer borders of the upper canine (Fig. iv).

M^D-M^D : posterior palatal width - taken across the outer borders of the last upper molar (Fig. iv).

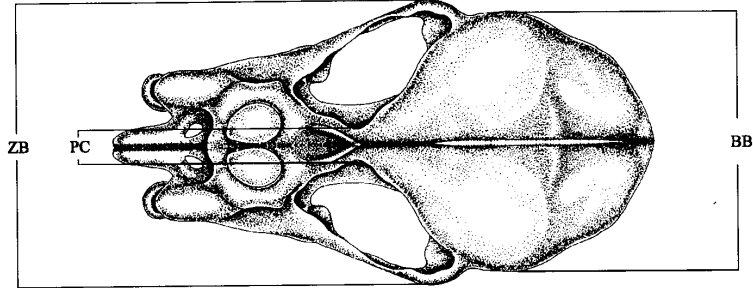


Fig. iii. Dorsal view of the skull of *Rhinolophus beddomei*.

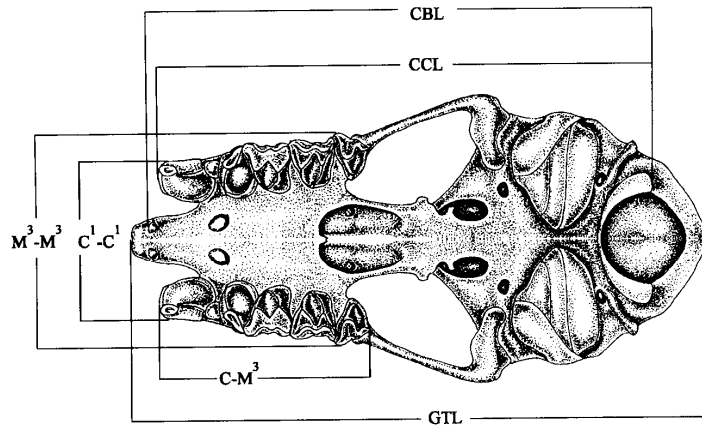


Fig. iv. Ventral view of the skull of *Rhinolophus beddomei*.

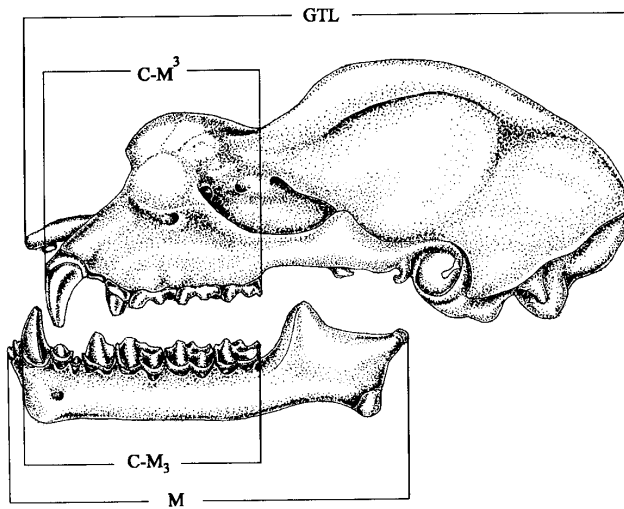


Fig. v. Lateral view of the skull of *Rhinolophus beddomei*.

Preparing wet specimens

Wet specimens

Specimens which are to be stored in alcohol should already have a field number on a field label (see above) attached to the right hind foot. They need to

- Be measured or have measurements transferred on to the specimen label⁴, which will eventually be attached to the bat's skull
- Have their collection data transferred on to a strong, tear proof, waterproof label⁷, which will be attached to the right foot of the wet specimen (the original field label should not be removed and therefore two labels should be attached to each wet specimen) and on to the skull label
- Have the skull extracted⁸, cleaned¹⁰ and labelled
- The identification confirmed and recorded on the label
- The collection number⁵ recorded on the label, which is attached to the right foot of the wet specimen

Skull label⁴

- Should be of durable but thin card or reinforced paper (such as Forbon Tags, 3-9/16" x 11/16")
- Have a thread for attaching to the mandible and cranium (thread – Button twist – Gutermann – CA-02776, polyester)
- Have the following information
 - Original field number (on back of label)
 - Collection number⁵, which is referable to a computerized or card index cataloguing system⁶ (on front of label)
 - Species name (on front of label)
 - Sex (on front of label)
 - Locality in full (on front of label)
 - Geographical co-ordinates (on front of label)
 - Collector(s) – if known (on front of label)
 - External measurements and weight (on back of label)
 - Any additional comments about the habitat, behaviour... (on back of label)

Harrison Institute No: HZM.2.35540

Rhinolophus malayanus sex ♂

○ 12.06.2005 Saddan-Sin Cave, 10 km north of Mawlamyine, Mon State, Myanmar, 16°44.01'N, 97°43.23'E.
Coll. Si Si Hla Bu & Paul Bates

Above: front of the skull label

Field No: PB120605.1

HB: 44.3 mm Tail: 22.9 mm FA: 41.3 mm

HF: 7.4 mm E: 16.9 mm Mass: 6.8

In a colony of over 100 horseshoe bats roosting in a side chamber of this large cave system

Below: back of the skull label

Collection number⁵

Each specimen in a zoological collection should have its own unique collection number:

- To ensure that the specimen can be accessed on the collection's database

- To ensure that different parts of the original specimen (for example, skull, body, baculum...) can be reliably linked together
- So that particular specimens can be described or referred to in publications.

Each collection tends to have its own system for the coding.

- Most have the initials of the Institution as the prefix letters, for example FMNH (Field Museum of Natural History) or AMNH (American Museum of Natural History).
- These letters are followed by a number. This may start as '1' and increase incrementally as each new specimen is added, for example FMNH.1, FMNH.2,...
- Others have a system that links the number of specimens with their date of registration in to the collection, for example BMNH.45.2005 which means the 45th specimen catalogued in the year 2005 in (the mammal section of) the British Museum of Natural History.

Each institution should choose its own method but it is essential that every zoological collection must devise and maintain a system of collection numbers and an associated catalogue. This maximises the future security of the specimens and greatly increases the value of the collection.

Catalogue⁶

Each specimen in a zoological collection should be catalogued. It should also be remembered that

- All specimens, once researched, should be donated to a permanent collection. This is essential if the specimen has been referred to in a publication (international or otherwise)
- Such a collection might be in a university, institute or a natural history museum
- Collections should be for the benefit of all researchers and conservationists
- Unless being actively researched, a collection should not be stored for private use only (ie not open to other researchers) – once that research is completed it should be donated elsewhere

In the past, most collections were on a simple card index system. Today new (or small) collections have a great opportunity to have their databases on computer.

- Each specimen has its own unique entry in a catalogue. It should include all the information that is written on the specimen label, namely:
 - Original field number
 - Collection number⁵
 - Species name
 - Sex
 - Locality in full
 - Geographical co-ordinates
 - Collector(s) – if known
 - External measurements and weight
 - Any additional comments about the habitat, behaviour...

Wet specimen label⁷

- Should be of strong, tear proof, waterproof card (ideally, Resistall Paper, 120 gms)
- Have a thread for attaching to the right hind foot of the specimen
- Have the following information

- Original field number
- Collection number⁵, which is referable to a computerized or card index cataloguing system⁶
- Species name
- Sex
- Date of collection
- Locality in full
- Geographical co-ordinates

Above: front of the wet specimen label

HZM.2.35540
Rhinolophus malayanus sex ♂
○ 12.06.2005 Saddam-Sin Cave, 10 km north of Mawlamyine, Mon State, Myanmar, 16°44.01'N, 97°43.23'E.

Below: back of the wet specimen label

○ PB120605.1

Notes:

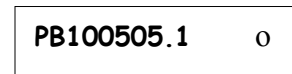
Skull extraction⁸

Extracting a skull

- A cut should be made from the corner of the mouth on one cheek to enlarge the hole through which the skull will be extracted
- The facial skin should be peeled back from the mandible and rostrum using a combination of forceps, small blunt scalpel and small sharp scissors
- When cutting the skin free from the nasal bones, it is important to avoid damaging any noseleaf or other interesting appendage, if present
- Care must also be taken not to cut or otherwise damage the zygomatic arches
- If the bat species has large eyes, care must be taken when cutting around the eyelids to ensure that the skin does not have unnaturally large openings
- To free the ears, the auditory meatus must be cut on each side of the skull. This should be done at a point as near the surface of the skull as possible to avoid making an unnecessary hole in the head skin.
- When cutting the skull free, it is important to cut the upper cervical spine rather than risk cutting the occipital part of the skull
- A temporary skull label¹⁰ with the field number (which corresponds to that on the leg of the wet specimen) should be immediately attached to the skull to ensure that there is no subsequent confusion between skulls

Temporary skull label¹⁰

- A simple tag label of fairly durable card
- Essential information to be recorded on the label is the field number
- This must be written in Indian/ permanent ink or pencil.



Temporary skull label with field number

Skull cleaning – manual dissection⁹

- The extracted skull, with its temporary skull label attached, should be suspended in water that is gently brought to the boil (instant immersion in boiling water may cause the skull to crack)
- The skull should remain in the simmering water for about 15 minutes for small skulls (*Pipistrellus*), 20 minutes for medium sized skulls (*Cynopterus*) and 30 minutes for large skulls, such as those of *Pteropus*. The length of time varies slightly on the method of preservation, number of years the skull has been in preservative... The technician needs to adjust times according to results.
 - If the skull has been boiled for too short a time, the muscle will be difficult to remove and damage may be caused to the skull
 - If the skull has been boiled for too long a time, the skull will tend to fall apart along suture lines
- Once boiled, the skulls should be left to cool down in the air and then stored temporarily in cold water (after 24-36 hours they begin to smell, longer than that and putrefaction sets in which will damage the skulls) until they are cleaned
- Small skulls should be cleaned under a dissecting microscope
- The tongue should be removed by cutting around the inside of the mandible (when viewed from below) and extracting the tongue with a pair of forceps
- The muscle on the braincase should be peeled off

- The mandible can then be teased away from the braincase – care should be taken not to break the zygomatic arches
- The muscle should then be removed from all parts of the skull using a combination of small, very fine pointed forceps, small scalpels and scissors
- Care must be taken not to damage the fine bones of the skull such as the hamular processes and the post palatal spine (when present)
- The brain should be removed through the foramen magnum using a hooked probe, cotton wool and forceps
- Once completely clean, the skull should be pinned out to dry in a specimen box with its label carefully pinned alongside it and left until it is dry

Skull cleaning – Dermestid beetle^{9b}

- If a colony of dermestid beetles is available this can be used to produce exceptionally well cleaned skulls and other osteological material. However, great care must be taken to ensure that the beetles do not escape into the main collection. Details of this method are included in Appendix 1.

Skull storage

- Once cleaned (and dry) the skull should have its skull label⁴ attached
- The label thread should pass either side of the postorbital constriction, within the zygomatic arches. It should not be tied tight to the skull but a knot should be tied that allows the skull to ride easily up and down the thread.
- The mandible should be tied on to the end of the thread, so that the label, cranium and mandible are all attached to each other
- The skull with its label should be stored in a small plastic pot with a secure lid
- The skull can be wrapped in tissue paper or supported on cotton wool to minimise any damage during storage.

Storing wet specimens

- Prepared specimens should be stored in air-tight jars
- Each jar should contain the specimens of only one species (ie all specimens of *Pipistrellus javanicus* should be in one (or more) jar/s; all specimens of *P. tenuis* in another, irrespective of whether the collecting localities are the same or different). The species name should be on a label attached to the outside of the jar
- Each specimen should be identified and with its data included on a label⁷, which is attached to the bat's right foot
- Jars should not be overfilled with specimens (crowding can damage/squash soft tissues such as delicate noseleaves)
- Jars should be air-tight. Simple, inexpensive jars such as old coffee jars are quite acceptable if the budget does not permit the purchase of specially made containers. However, it is essential that the alcohol cannot evaporate through the lid or lid seal
- Jars should contain 70% ethyl alcohol (and be regularly checked to ensure that the specimens remain below the level of the alcohol)
- Jars should be kept in the dark (never on open shelves in light [or even worse sunlight], as the specimens will quickly fade and become a uniform pale brown); if cabinets with glass doors are all that is available, black card or paper should be attached to the glass to eliminate as much light as possible from reaching the specimens.

Preparing a bat study skin

Dry study skin

Prepared dry skins are particularly valuable for the study of hair colour. They are poor for the study of soft tissue parts such as noseleaves or the structure of the ear. They are also exceptionally difficult to store in the tropics or in any area with high humidity.

- To make the first incision, pinch the skin of the upper abdomen between the fingers of one hand so that it is separated from the muscular body wall underneath. Make a longitudinal cut through the skin to a point just in front of the anus, being careful not to cut into the abdominal wall
- In males evert the penis and sever this organ between the skin and body as close to the body wall as possible
- Expose the knee joints and cut through the knees
- At all times, use cornmeal or powder to absorb any excess blood
- When both legs are free cut through the rectum and tail to free the skin from the abdomen
- Using forceps pull out the tail
- Work the skin loose from the body – to avoid stretching the skin always pull the body from the skin and not vice versa
- Release the wings in the same way as the legs but cut through the humerus above the elbow joint to ensure the forearm measurement can be taken
- Work forward until the bases of the ears are exposed
- Cut the auditory canal as close to the entrance of the skull as possible
- When both ears are free, work forward cautiously until the posterior edges of the eyes can be seen through a layer of transparent tissue
- Hold the skin slightly away from the head and cut through this membrane just over the eye. Take care not to cut through the eyelids
- Carefully cut the attachment of the skin at the front corner of the eye
- Work forward until the lips are reached.
- Using scalpel or scissors free the lips from the bone
- Cut the nasal cartilage to release the skin from the body
- Lay the skin on a clean surface and remove any pieces of flesh or blood
- Wrap cotton wool around the lower leg bones and turn the legs the right side out
- Make a small ball of loose and fluffy cotton wool to act as the head and body
- Invert the skin and using fine forceps slide the apex of the cotton wool ball into the head skin
- Slide a thin (very thin) straight wire into the tail; the wire should exceed the length of the tail and provide extra support for the body
- Arrange and tidy up the wool body and then sow up the incision with a needle and black cotton
- Sow up the mouth with two separate stitches
- Tie the specimen label to the right hind foot
- Pin out the specimen
 - Wings folded alongside the body with the wrist level with the nose
 - The phalanges and metacarpals are spread just enough so that each bone is accessible for measuring
 - The thumb is pinned alongside the second metacarpal

- The feet are pulled back and out but not spread further than the pinned position of the wings
- A pin is put behind each calcar to draw the membrane tight

Preparing a baculum

The baculum is a bone in the penis of some species of bat that in certain taxa, for example the genus *Pipistrellus*, can be a very important character for identification. To prepare a baculum

- Remove the penis by cutting off the organ as close to the surface of the body as possible – otherwise the baculum may be damaged
- Place in a small glass or plastic tube with a secure lid
- Ensure that the tube is labelled with the field number
- Remove the penis from the plastic tube with forceps and place it in a test tube half filled with cold water and bring to the boil
- Simmer for two minutes
- Invert the test tube on to a very fine wire mesh suspended over a beaker, so that the penis is easily visible
- Half fill the original plastic tube with 5% KOH (potassium hydroxide) and a pinch of alizarin red powder – this latter substance stains the baculum
- Place the baculum in the tube and leave for 24 hours
- Dissect out the baculum from the tissue under a dissecting microscope with very fine forceps
- Wash out the plastic tube and half fill with glycerine
- Carefully place the baculum in the tube
- Ensure that the tube is labelled with the following information
 - Original field number
 - Collection number⁵, which is the same as that of the body and skull
 - Species name
 - Date of collection
 - Locality and geographical co-ordinates
- Store in an upright position

HZM.2.35540

Rhinolophus malayanus sex ♂

- 12.06.2005 Saddam-Sin Cave, 10 km north of Mawlamyine, Mon State, Myanmar, 16°44.01'N, 97°43.23'E.

PB120605.1

An example of the front and back of a label to be attached to a baculum tube.

Appendix 1

Wet specimen labels – Resistall Paper 8.5”x11” 120 gsm (for laser printer) [order number: L219-368511]^a

Skull labels – Forbon Tags 3-9/16”x 11/16” [order number: L217-4916]^a

Pen for writing on labels – Pigma Micron 05^a

Pen for writing on skulls – Pigma Micron 005^a

Appendix 2

Equipment required for baculum study

- Small plastic/glass tubes^b
- Glycerin
- Alizarin red powder
- Potassium hydroxide solution 5%
- Forceps – ideal-tek 5.s^b
- Card labels
- Thread
- Indian ink Rotring pen and black ink (never biro)

Equipment for prepared skull storage

- Small plastic pots^b
- Cotton wool
- Card labels
- Thread
- Indian ink Rotring pen and black ink (never biro)

Equipment for storing wet specimens

- Dark storage cabinets
- Storage Jars (with tight fitting lids)
- 70% ethyl alcohol
- Jar labels (Dymo or paper)
- Wet specimen labels
- Pens for writing on labels – Pigma Micron 05^a or Indian ink

Suppliers of equipment in the UK

^a : Preservation Equipment Ltd, Vinces Road, Diss, Norfolk IP22 4HQ, UK

Tel: + 44 (0)1379 647400, E-mail: sales@preservationequipment.com

<https://www2.preservationequipment.co.uk>

^b: Watkins and Doncaster, P.O. Box 5, Cranbrook, Kent, TN18 5EZ, UK.

Tel: + 44 (0)845 8333133; Fax: + 44 (0)1580 754054, E-mail: sales@watdon.com

www.watdon.com