VI. Material and Methods

The comparative investigations concentrate on marsupial and placental species with different habitats and lifestyle, but with a body size comparable to that of *Henkelotherium guimarotae*.

The skeletons used for comparisons come from the following institutions (for which the following abbreviations are used): MfN or ZMB, Museum für Naturkunde, Berlin; SMF or SM, Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main; IP-FUB, Institut für Paläontologie, (actually named Institut für Geowissenschaften – Fachrichtung Paläontologie-), Freie Universität, Berlin; MNCN Museo Nacional de Ciencias Naturales, Madrid, Spain; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA.

The two living specimens of *Monodelphis domestica* used for radiographic film and the dissections come from the Museum für Naturkunde, Berlin (Museum of Natural History) of the Humboldt Universität zu Berlin, where they are breed as laboratory animal. The killing of the two specimen of *Monodelphis domestica* for the purpose of dissection is officially reported with Reg.-number 0253/99 at the Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit Berlin. The specimen of *Micromys minutus* used in the cineradiographic study come from a breed of doctorate students of the Museum für Naturkunde Berlin.

The species are listed in tables and figures after systematic position and size criteria: first to the left (or top) *Henkelotherium guimarotae* is presented followed by marsupial and subsequently placental species, with their respective sizes increasing from left to right (or top to bottom).

List of skeletons studied:

Species	Family	English name	Specimen number
Henkelotherium guimarotae	Paurodontidae		IP-FUB Gui Mam 138/76
MARSUPIALIA			
Tarsipes spenserae	Tarsipedidae	Honey possum	ZMB 3320
Sminthopsis crassicaudata	Dasyuridae	Fat-tailed Sminthopsis	ZMB 32389
Monodelphis domestica	Didelphidae	Pygmy Opossum	MfN 748 f
Monodelphis sorex	Didelphidae		ZMB 35515
Marmosa sp.	Didelphidae	South American Mouse Opossum	IP-FUB 26
PLACENTALIA			
Micromys minutus	Muridae	Old World Harvest Mouse	ZMB 74195
Sorex araneus	Soricidae	Common Shrew	IP-FUB 2
Muscardinus avellanarius	Gliridae	Dormouse, Hazel Mouse	ZMB 96254
Acomys cahirinus	Muridae	Egyptian Spiny Mouse	SM-33120
Mus musculus	Muridae	House Mouse	ZMB 65
Apodemus agrarius	Muridae	Field Mouse	ZMB 5035
Apodemus sylvaticus	Muridae	Wood Mouse	ZMB 74161
Neomys fodiens	Soricidae	Water Shrew	ZMB 16975
Elephantulus brachyrhynchus	Macroscelididae	Elephant Shrew	ZMB 84913
Microgale sp.	Tenrecidae	Shrew-like Tenrec	ZMB 71610
Galago demidovii	Lorisidae	Demidoff Bushbaby	IP-FUB 40
Microcebus murinus	Lemuridae	Mouse Lemur	IP-FUB 38
Ptilocercus lowii	Tupaiidae	Pen-tailed Tree Shrew	MCZ-51736
Talpa europaea	Talpidae	Common European Mole	ZMB
Galemys pyrenaicus	Talpidae	Pyrenean Desman	MNCN-3136
Tupaia sp.	Tupaiidae	Tree Shrew	IP-FUB 1
Saguinus oedipus	Callithricidae	Long-tusked Marmoset	IP-FUB 25
Rattus sp.	Muridae	Rat	IP-FUB 21
Sciurus vulgaris	Sciuridae	European Squirrel	IP-FUB 28

The following skeletons from Museum für Naturkunde, Berlin, were used for the comparison of the number of vertebrae (Table 1): *Monodelphis* sp. ZMB 35516, *Metachirus nudicaudatus* ZMB 81050, *Gracilianus* sp. ZMB 7262, *Acomys cahirinus* ZMB 15400, *Microgale sp.* ZMB 71614. Several skeletons of the squirrel

species *Sciurus carolinensis* and *Sciurus niger* were studied at the collections of the Museum of Comparative Zoology of the Harvard University (USA) in order to test the presence of tubercula in the ventral side of their phalanges.

1. Morphological comparisons and osteometry

Osteometric data from *Henkelotherium* and Recent marsupials and placentals were obtained using a Reflex-Microscope/Peterson Electronics Ltd., which allows measuring the objects without contact (theoretical measuring accuracy of 2µm). The human measuring error: aproximately 0,2 mm. The data were processed with the software program C3D® which calculates distances between selected points. One species (*Ptilocercus lowii*) was manually measured in the MCZ at the Harvard University (USA) with a caliper (Electronic Digital Caliper W®).

The length of the skeletal elements that were measured in the osteometric studies is the maximal length, as in similar osteometric studies (Jouffroy 1984). Except for the scapula where the functional length was measured (middle of glenoidal cavity to end of the spine) (Duerst 1930). The measurements were realized using the methods and skeletal measuring points of Duerst (1930) and Knußman (1998).

The osteometric data collected from all these small mammalian species include absolute measurements (in mm) of skeletal elements and relative indices to facilitate interspecific comparisons in relation to body length. The length of the last 7 presacral vertebrae was selected as an indicator of body length because it is the only portion of the vertebral column which is well preserved and still articulated in *Henkelotherium*.

The relative indices in relation to body length were calculated by dividing the absolute measurements by the body length (atlas to sacrum) of each specimen. Thus, it was possible to compare and to check (Figs. 1-5) the variaton in the relative data obtained by dividing by the two references of body length used: i) the length of the last 7 presacral vertebrae, ii) the body length (atlas to end of sacrum).

Regression curves, diagrams, graphics were calculated by means of the software Excel® of Microsoft®.

For the drawings of skeletal elements and joint geometries a Zeiss® camera lucida was used.

2. Muscular dissections

Macroscopic dissections of the abdominal, inguinal, thing and forearm musculature of two specimens: a male (MfN m-699), and a female (MfN f-748) of *Monodelphis domestica*, were carried out. *M. domestica* was chosen because it is a generalist marsupial species of small size (exceeding the size of *Henkelotherium* by 50-70%). With respect to the general plan of their skeletons there is a high degree of similarity (e.g. skeletal proportions, presence of epipubes) between *Henkelotherium guimarotae* and *Monodelphis domestica*. A detailed comparison of the preserved skeletal parts of *Henkelotherium guimarotae* with those of *Monodelphis domestica* allows for a reconstruction of the origins and insertions of some elements of the fossil's muscular apparatus (e.g. in the inguinal region).

Dissections were carried out with the specimen permanently submerged in water which yields more detailed results than by conventional dissection in air (Frey 1988, Frey and Hofmann 1998). Overnight the specimen was kept at 0-4 °C.

Some droplets of 5% formaldehyde were added to avoid bacterial growth. Dissection stages were documented photographically and the origin, insertion and other characteristics of each muscle were described. Subsequent to the dissection, the specimens were macerated to provide skeletal elements for further comparative investigation.

3. Cineradiographic analysis and reconstruction

One marsupial, *Monodelphis domestica,* and one placental, *Micromys minutus,* were radiographed while ascending vertically on a flat and on a round wooden substrate. Images were recorded at the Institut für den Wissenschaftlichen Film (IWF) at Göttingen, Germany. The X-ray system consists of an automatic Phillips® - Camsys+ unit with a single X-ray source image amplifier chain (Shilling and Fischer 1999). Pulsed X-ray exposures were applied (50 Kv, 200mA) to film at 150 frames/s. The images were taken from the image amplifier using a Arritechno® R 35-150 camera. X-ray films were copied to video tapes (VHS). The cineradiographic tapes were A-D converted with a video processing board (Screen Machine® I, Fast® Multimedia AG, Munich, Germany). The frames were further processed at ISZ Jena, by using a software that was written for this specific purpose (“Unimark” by R. Voss). It allows to digitize interactively previously defined landmarks with a cursor function, to correct distorsion automatically, to calculate angles and distances, and to correct easily erroneously digitized coordinates during analysis (Schilling and Fischer 1999). Skeletal landmarks were fixed and their x-y coordinates saved for each frame. The coordinates were used to define vectors and to calculate angles between vectors. Angles were defined anatomically (e.g. knee joint: femur-tibia), or calculated

against the vertical plane (e.g. humerus-vertical) (Fischer 1998, Schilling and Fischer 1999).

The cineradiographic studies were realized in cooperation with the ISZ-Jena. A vertical ascending sequence of *Monodelphis domestica* composed of 144 images was analyzed. Angles between extremities and angles between bones and the vertical were calculated in each frame. The results of this study were compared with others studies (Schilling and Fischer 1999, of *Tupaia glis*; Freytag S. unpublished data of *Acomys cahirinus*) which had used the same technique to analyze the horizontal locomotion of small sized mammalian species, including *Monodelphis domestica* (Kühnapfel 1996). The body posture, the angles between limb segments and the relative positions of skeletal parts in the reconstruction of *Henkelotherium*, are based on the data obtained by cineradiographic analysis of *Tupaia glis* (Schilling and Fischer 1999) and *Monodelphis domestica*.

4. Abbreviations:

C	Cervical vertebrae
C1	Atlas (first cervical vertebrae)
Carp.	Carpus
Cp. femur	Caput femoris
Cnd. hum.	Condylus humerii
Cond. lat.	Condylus lateralis ossis femoris
Cond. med.	Condylus medialis ossis femoris
Cond. rad.	Condylus radialis
Cond. uln.	Condylus ulnaris
Cont. thor.	Contornus thoracicus

Cost.	Os costae
CR	Skull
Epicond. med.	Epicondylus medialis humeri
Epip.	Epipubes
Fib.	Fibula
Fem.	Femur
Fos. isp.	Fossa infraspinata
Fos. ssp.	Fossa supraspinata
Hum.	Humerus
L	Lumbar vertebrae
Mtcarp.	Metacarpus
Mttars.	Metatarsus
M. grac.	Musculus gracilis
Mm. adduct.	Musculi adductores
M. obl. ext.	Musculus obliquus externus abdominis
M. obl. int.	Musculus obliquus internus abdominis
M. pyr.	Musculus pyramidalis
M. pect.	Musculus pectineus
M. rect. abd.	Musculus rectus abdominis
Os mars.	Epipubes (Os marsupium)
Pelv.	Pelvis
Phal.	Phalanx
Rad.	Radius
S	Sacrum
Scap.	Scapula
Symph. pelv.	Symphysis pelvina

Ster.	Sternum
T	Thoracic vertebrae
Tars.	Tarsus
Tib.	Tibia
Tr. Maj.	Trochanter major
Trochl.	Trochlea humeri
Tuberc.	Tubercula
Uln.	Ulna
Vag. m. rect. abd.	Vagina musculi rectus abdominis

The segments of the vertebral column are named using the following abbreviations: C, cervical; T, thoracic; L, lumbar; S, sacrum; CD, caudal vertebrae (tail).

The following abbreviations of institutional names are used:

DFG, Deutsche Forschungsgemeinschaft; MfN or ZMB, Museum für Naturkunde, Humboldt Universität, Berlin; SMF or SM, Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main; IP-FUB, Institut für Paläontologie, (actually named Institut für Geowissenschaften – Fachrichtung Paläontologie-), Freie Universität, Berlin; ISZ, Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum, Friedrich-Schiller-Universität, Jena, Germany; IWF, Institut für den Wissenschaftlichen Film, Göttingen; IZW, Institut for Zoo Biology and Wildlife Research, Berlin; MACN, Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires, Argentina; MNCN Museo Nacional de Ciencias Naturales, Madrid, Spain; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA.