### **Supporting Information**

*Anatomical diversification of a skeletal novelty in bat feet* Kathryn E. Stanchak, Jessica H. Arbour, Sharlene E. Santana

This document includes:

Appendix S1 – Specimen list for  $\mu$ CT scanning and histology with CT scanner settings and anatomical descriptions.

Additional Supporting Information includes:

LengthMeasurementSpecimens.csv (a list of specimens from which we compiled our calcar length data)

CalcarLengthData.csv (data set used in calcar length macroevolutionary analyses)

Institution Acronyms in List of Specimens: AMNH: American Museum of Natural History Mammal Collection FMNH: Field Museum of Natural History Mammal Collection Lab: Santana Lab in the Department of Biology at the University of Washington MCZ: Museum of Comparative Zoology Mammal Collection MVZ: Museum of Vertebrate Zoology Mammal Collection NMNH: United States National Museum Mammal Collection at the Smithsonian National Museum of Natural History PSM: Slater Museum of Natural History Mammal Collection

#### Please see manuscript for full description of methods.

## **APPENDIX S1**

CT scanning and histology specimen list with anatomical descriptions of calcar and ankle modifications. (Note that because of the limited number of individuals per species in our sample, we were unable to confirm absence of a synovial joint in the histological sections.)

Artibeus jamaicensis (Phyllostomidae) – Santana Lab 022614-25

Procedures: CT scanning and histological sectioning.

Small calcified nodule of cartilaginous calcar near articulation with calcaneus. No evidence of internal structure (i.e., not bony). Synovial joint between calcar and calcaneus. Sesamoid on plantar side of tibia, just above astragalus.

Scanned on Skyscan1172 at 8.88µm, 60kV, 167µA.

*Balantiopteryx plicata* (Emballonuridae) – Santana Lab 0229-076 Procedures: CT scanning and histological sectioning.

Long calcified portion of calcar with less densely-calcified or uncalcified interior. Calcar is entirely cartilaginous. More densely calcified "cap" to calcar at articulation with calcaneus. Sesamoid on plantar/lateral side of lateral cuneiform (the cuneiform above MT1).

Scanned on Skyscan1172 at 7.97µm, 60kV, 167µA.

*Cynopterus brachyotis* (Pteropodidae) – Lubee Bat Conservancy

Procedures: CT scanning, diceCT scanning, dissection, and histological sectioning.

Uncalcified cartilage calcar not contiguous with the tendon of the *m. gastrocnemius*. Sesamoid on plantar/lateral side of lateral cuneiform.

Scanned on Skyscan1172 at 10.58µm, 50kV, 200µA. diceCT scan on Skyscan1172 at 10.58µm, 40kV, 250µA after 2 days in 3% (total solute) Lugol's iodine.

Desmodus rotundus (Phyllostomidae) - Santana Lab 022414-06

Procedures: CT scanning and histological sectioning.

Calcar has small, bony nodule with clear internal structure but is distally cartilaginous. Synovial joint between calcar and calcaneus. Middle cuneiform is contiguous with/fused to the navicular. Sesamoid on plantar side of lateral cuneiform and the middle cuneiform/navicular fusion. Sesamoid on plantar side of MT5.

Scanned on Skyscan1172 at 12.67µm, 50kV, 200µA.

Eptesicus fuscus (Vespertilionidae) - Santana Lab KES 037

Procedures: CT scanning and histological sectioning.

Cartilaginous calcar has lightly calcified section and an uncalcified "keel" hook. Synovial joint between calcar and calcaneus. Sesamoid on dorsal side of astragalus. Sesamoid on anterior/lateral side of lateral cuneiform.

Scanned on Skyscan1172 at 11.50µm, 40kV, 250µA.

Furipterus horrens (Furipteridae) - AMNH 142903

Procedures: CT scanning (whole specimen).

Long, calcified cartilage calcar with no evidence of internal bony structure. Sesamoid above (superior to) lateral cuneiform.

Scanned on Skyscan1173 at 12.14µm, 70kV, 114µA.

*Glossophaga longirostris* (Phyllostomidae) – Santana Lab GR110 Procedures: CT scanning and histological sectioning.

Uncalcified, cartilaginous calcar. Sesamoid on plantar side of tibia and astragalus. Scanned on Skyscan1172 at 9.93µm, 60kV, 167µA.

Hipposideros armiger (Hipposideridae) - AMNH 272276

Procedures: CT scanning and histological sectioning.

Uncalcified cartilaginous calcar (but with perhaps a slight calcified band just above calcaneus). Sesamoid on dorsal side of astragalus. Sesamoid on plantar/lateral side of lateral cuneiform. Sesamoid on plantar side of proximal end of 5<sup>th</sup> metatarsal. Scanned on Skyscan1172 at 9.80µm, 50kV, 200µA.

Macrotus waterhousii (Phyllostomidae) - Slater Museum FHA135

Procedures: CT scanning and histological sectioning.

Calcar is primarily bony and forms synovial joint with calcaneus. Sesamoid on plantar/lateral side of middle cuneiform.

- Scanned on Skyscan1172 at 8.88µm, 60kV, 167µA.
- Megaderma spasma (Megadermatidae) FMNH 205388

Procedures: CT scanning and histological sectioning.

Uncalcified cartilaginous calcar (but with perhaps a slight calcified band just above calcaneus). Calcar forms synovial joint with calcaneus. Sesamoid on plantar/lateral side of lateral cuneiform. Sesamoid on plantar side of proximal end of 5<sup>th</sup> metatarsal. Scanned on Skyscan1172 at 9.80μm, 50kV, 200μA.

Molossus molossus (Molossidae) – Slater Museum FHA1857

Procedures: CT scanning and histological sectioning.

Calcified calcar with internal bony structure at proximal end; cartilage distally. Calcar forms synovial joint with calcaneus. Sesamoid on dorsal side of astragalus. Sesamoid on plantar/lateral side of lateral cuneiform. Sesamoid on plantar side of middle and lateral cuneiforms.

Scanned on Skyscan1172 at 10.71µm, 60kV, 167µA.

Myotis californicus (Vespertilionidae) - Santana Lab KES 026

Procedures: CT scanning and histological sectioning.

Calcified cartilage calcar that forms an uncalcified "keel" hook. Calcar forms synovial joint with calcaneus. Sesamoid on plantar/lateral side of lateral cuneiform.

Scanned on Skyscan1172 at 10.71µm, 40kV, 250µA.

Mystacina tuberculata (Mystacinidae) - MVZ 173918

Procedures: CT scanning (whole specimen).

Calcified calcar with internal structure that appears bony at proximal end. Calcification forms two long "tines". Sesamoid on plantar/lateral side of lateral cuneiform.

Scanned on Skyscan1173 at 20.96µm, 60kV, 133µA.

Natalus mexicanus (Natalidae) – Santana Lab 0229-02

Procedures: CT scanning and histological sectioning.

Calcified cartilage calcar with less densely-calcified or uncalcified interior. Calcar forms synovial joint with calcaneus. Medial and middle cuneiforms appear fused. Sesamoid on dorsal side of distal end of astragalus/proximal end of fused medial and middle cuneiforms.

Scanned on Skyscan1172 at 6.92µm, 60kV, 167µA.

Noctilio leporinus (Noctilionidae) - Slater Museum FHA1651

Procedures: CT scanning and histological sectioning.

Calcar has long calcified section that has distinctly bony internal structure, including what appear to be small trabeculae. Sesamoid on plantar side of lateral and middle cuneiforms. Sesamoid on plantar side of proximal end of 5<sup>th</sup> metatarsal.

Scanned on Skyscan1172 at 12.15µm, 60kV, 167µA.

Nycteris hispida (Nycteridae) - USNM 478968

Procedures: CT scanning and histological sectioning.

Long, calcified cartilage calcar with less densely-calcified or uncalcified interior. Cuboid, medial cuneiform, and middle cuneiform are fused. Sesamoid on plantar side of lateral cuneiform.

Scanned on Skyscan1172 at 6.88µm, 50kV, 200µA.

Pteronotus quadridens (Mormoopidae) - Slater Museum FHA 780

Procedures: CT scanning and histological sectioning.

Long, primarily calcified cartilage calcar with bony tissue at proximal end near articulation with calcaneus. Sesamoid on plantar side of lateral cuneiform. Sesamoid on plantar side of proximal end of 5<sup>th</sup> metatarsal. Sesamoid on dorsal/medial side of cuboid. Scanned on Skyscan1172 at 6.92µm, 60kV, 167µA.

## Pteropus sp. (Pteropodidae) - Herring Lab.

Procedures: CT scanning, diceCT scanning, dissection.

Uncalcified cartilage calcar that is contiguous with the tendon of the *m. gastrocnemius*. Scanned on Skyscan1172 at 26.44µm, 50kV, 200µA. diceCT scan on Skyscan1172 at 26.44µm, 40kV, 250µA after 3 days in 3% (total solute) Lugol's iodine.

# Rhinolophus affinis (Rhinolophidae) - AMNH 234034

Procedures: CT scanning and histological sectioning.

Uncalcified cartilaginous calcar. Sesamoid on dorsal side of distal end of astragalus. Scanned on Skyscan1172 at 6.40µm, 50kV, 200µA.

### Rhinopoma hardwickii (Rhinopomatidae) - FMNH 123185

Procedures: CT scanning and histological sectioning.

Uncalcified cartilaginous calcar. Sesamoid on dorsal side of distal end of astragalus. Sesamoid on plantar side of navicular. Sesamoid on plantar side of proximal end of 5<sup>th</sup> metatarsal.

Scanned on Skyscan1172 at 7.45µm, 50kV, 200µA.

### Rousettus aegyptiacus (Pteropodidae) - Herring Lab

Procedures: CT scanning, diceCT scanning, dissection, and histological sectioning.

Uncalcified cartilage calcar that is contiguous with the tendon of the *m. gastrocnemius*. Sesamoid on plantar/lateral side of lateral cuneiform. Small calcified nodules on dorsal side of foot between proximal ends of calcaneus and astragalus.

Scanned on Skyscan1172 at 13.22µm, 50kV, 200µA. diceCT scan on Skyscan1172 at 10.18µm, 40kV, 250µA after 2 days in 3% (total solute) Lugol's iodine.

### *Thyroptera tricolor* (Thyropteridae) – MVZ 173918

Procedures: CT scanning (whole specimen/hindlimbs only).

Long, primarily uncalcified cartilage calcar calcified nodule at articulation with calcaneus. Calcar has two obvious uncalcified "keel"-like structures (based upon external examination of specimen). Navicular and middle cuneiform are fused. Sesamoid on plantar side of lateral cuneiform.

Scanned on Skyscan1173 at 13.15µm, 60kV, 133µA.