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A simple device to collect, store and study samples of two-dimensional spider webs

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Silk production and spinning are the most prominent characteristics of spiders (Araneae). The high diversity of web architectures correlates both with preying behaviors and with the ultra-structure of the silk threads that compose the webs, which in turn have a corresponding diversity of silk gland spigot complements. The details of web construction, thread structure, and gland spigot morphology have proven extremely informative for phylogenetic hypotheses (e.g., Eberhard 1982, 1988; Eberhard & Pereira 1993; Coddington 1989; Griswold *et al.* 2005; Hormiga *et al.* 1995; Lopardo & Ramírez 2007; Platnick *et al.* 1991; Agnarsson & Blackledge 2009; Eberhard 2010), and a fascinating system to study spider diversification and evolutionary trends (e.g., Opell 1996; Opell & Bond 2000; Blackledge *et al.* 2009, 2012; Dimitrov *et al.* 2012).

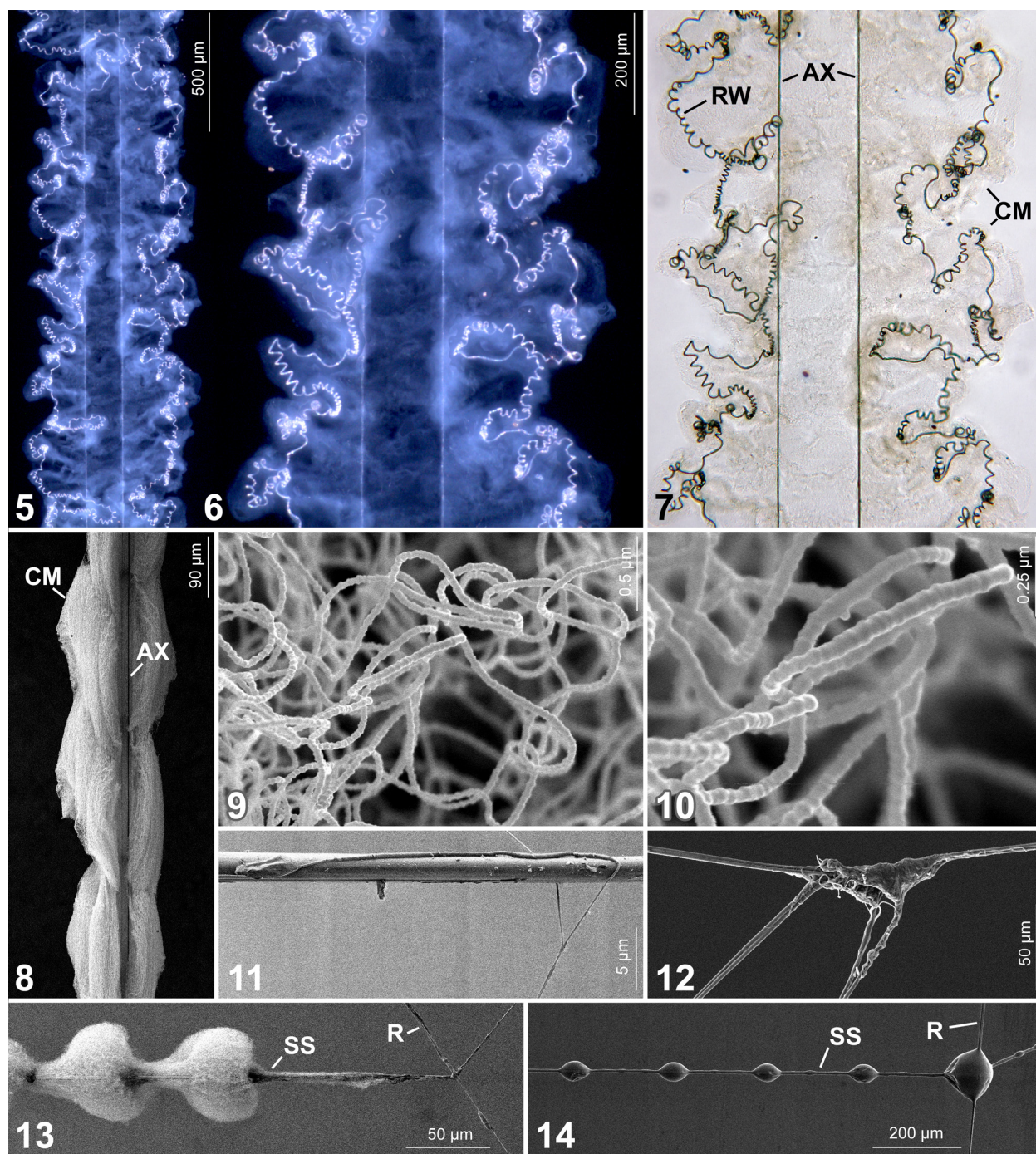
The architecture of a spider web can be documented with photographs, for which Carico's (1977) device made a significant progress, but the sampling of webs has several complexities, especially because of the stickiness of capture threads (Opell 1997), whose structure becomes altered upon the slightest contact. We have tested during several years a very simple device to collect and store two-dimensional web samples, which allows for easy observation using a light or scanning electron microscopy (SEM). Although comparable in concept, our devices are simpler to build than those designed by Opell & Hendrix (2009) and Opell *et al.* (2011) for single lines, and more importantly, the closed frame we implemented can hold a two-dimensional web without significant distortion. Here we describe this simple device along with some observation and imaging techniques, in the hope that it will promote the documentation of the high diversity of silk structures that are yet to be discovered, and thus facilitate studies that seek establishing a correlation of morphological structures with web building behaviors.



FIGURES 1–4. Silk samplers, boxes and specimen holder. 1, Silk sampler; 2, Small portable specimen box; 3, Specimen holder for SEM examination; 4, Slide box with silk samplers, for long-term storage.

Sampler preparation. The samplers are made with regular glass microscope slides, and a rectangular frame of double-sided poster tape (Fig. 1). This adhesive tape is made of synthetic foam about 1.5 mm thick, which suspends the silk sample, preventing it from touching the glass (for example, 3M™ Double Coated Urethane Foam Tape 4016 Off-White). The frame consists of four stripes of tape 3 mm wide. To build this frame, we stick one side of the tape first on a sheet of silicone coated paper (e.g., from a used sheet of adhesive labels) and make the 3 mm stripes using a cutter and a

ruler. The stripes are then adhered onto the microscope slide with the paper-covered sticky side facing up. One tip of the slide has space for an adhesive label, the opposite tip has space for manipulation or a further label, and the longer frame stripes are separated 0.5 mm from the edges of the slide to prevent the sampler from sticking to the containers. For very small webs (e.g., those of symphytognathoids) or for sampling a minute area of a web, a smaller frame can be simply adjusted by producing a smaller squared frame, or adding further transversal stripes inside the sampling rectangle.



FIGURES 5–14. Samples of silk. 5, 6, Cribellate silk band of *Progradungula otwayensis* Milledge 1997 (Gradungulidae), incident light in stereomicroscope; 7, Same, transmitted light in compound microscope; 8–10, Cribellate silk band of *Thaïda peculiaris* Karsch 1880 (Austrochilidae), SEM; 9, 10, Detail of cribellar fibrils; 11, Ampullate thread of *Austrochilus forsteri* Grismado, Lopardo & Platnick 2003 (Austrochilidae) with thinner threads of the kleptoparasite *Sofanapis antillanca* Platnick & Forster 1989 (Anapidae); 12, Junction of structural threads, *Scytodes* sp. (Scytodidae) from Monteverde (Costa Rica); 13, Cribellate spiral and radius, *Uloborus* sp. (Uloboridae) from KwaZulu-Natal (South Africa); 14, Viscid spiral and radius, *Nephila* sp. (Nephilidae) from KwaZulu-Natal (South Africa) the droplets of the sticky spiral are only slightly shrunk by evaporation. (AX = axial line; CM = cribellate mass; R = radius; RW = reserve warp; SS = sticky spiral.)

Collection and storage. At the moment of collection, the exposed side of the frame is peeled from the protective paper, and the sampler is gently pushed on the web, which immediately sticks on the adhesive surface. Once the contact with the adhesive is complete, the portion of the web outside the frame can be removed with the fingertips. While the softer webs made of thin threads (e.g., those of symphytognathoids or synotaxids) can be sampled directly, stronger webs (e.g., those of araneids or austrochilids) can slide through the sticky surface at the moment of taking the sample. For these, pressing the web with the fingertips against the sharp sides of the glass slide helps cutting the threads without distorting the structure. Once the sample is taken, a voucher code and the relevant data are written in the sampler label, a similar label is added to the vouchered specimen, and more detailed notes are taken in the field book if necessary. The silk samplers are conveniently carried (e.g., in the pockets of a collecting vest) using small five-slide containers with a lateral lid (Fig. 2; <http://www.emsdiasum.com> item #71550). Because the silk samplers are thicker than a regular glass slide, only two of them will fit in each of these boxes. Once the samples are taken and properly labeled, they can be stored in regular slide boxes, leaving one empty slot between samplers (Fig. 4). We have not detected any alteration in 15 year-old samples of webs of austrochilid spiders. Viscid silk however may alter its structure by evaporation in storage, and in the vacuum chamber of a standard SEM, although that effect may not be very drastic (Fig. 14).

Observation. While there is a wealth of information on web architecture, construction routines and morphology of spinnerets, the knowledge of the detailed composition of cables, junctions and web surfaces is still fragmentary (see Eberhard 2010). We provide here some images to illustrate that silk samples can be easily studied and provide informative data to fill in such gaps. Silk samples mounted as described above can be directly examined on the compound or stereo microscope (Figs. 5–7), or sputter-coated for SEM study (Figs. 8–14). For SEM observation, we use a flexible copper stripe of the same width of a glass slide, but slightly longer, so that the tips are bent about 5 mm over each extremity to hold the silk sampler. We glued two aluminum stubs on the back of the copper stripe, fitting the sample holder of the SEM (Fig. 3). The axial lines and reserve warp of cribellate bands are inside the cribellate mass, and thus can be better observed on a compound or stereo microscope (Figs. 5–7; see also Griswold *et al.* 2005: figs. 120–125). SEM observations can be used to reveal structural details, such as the surface of cribellate fibrils (Figs. 9, 10) and the composition of cables and junctions (Figs. 11–14).

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