

Supporting Information

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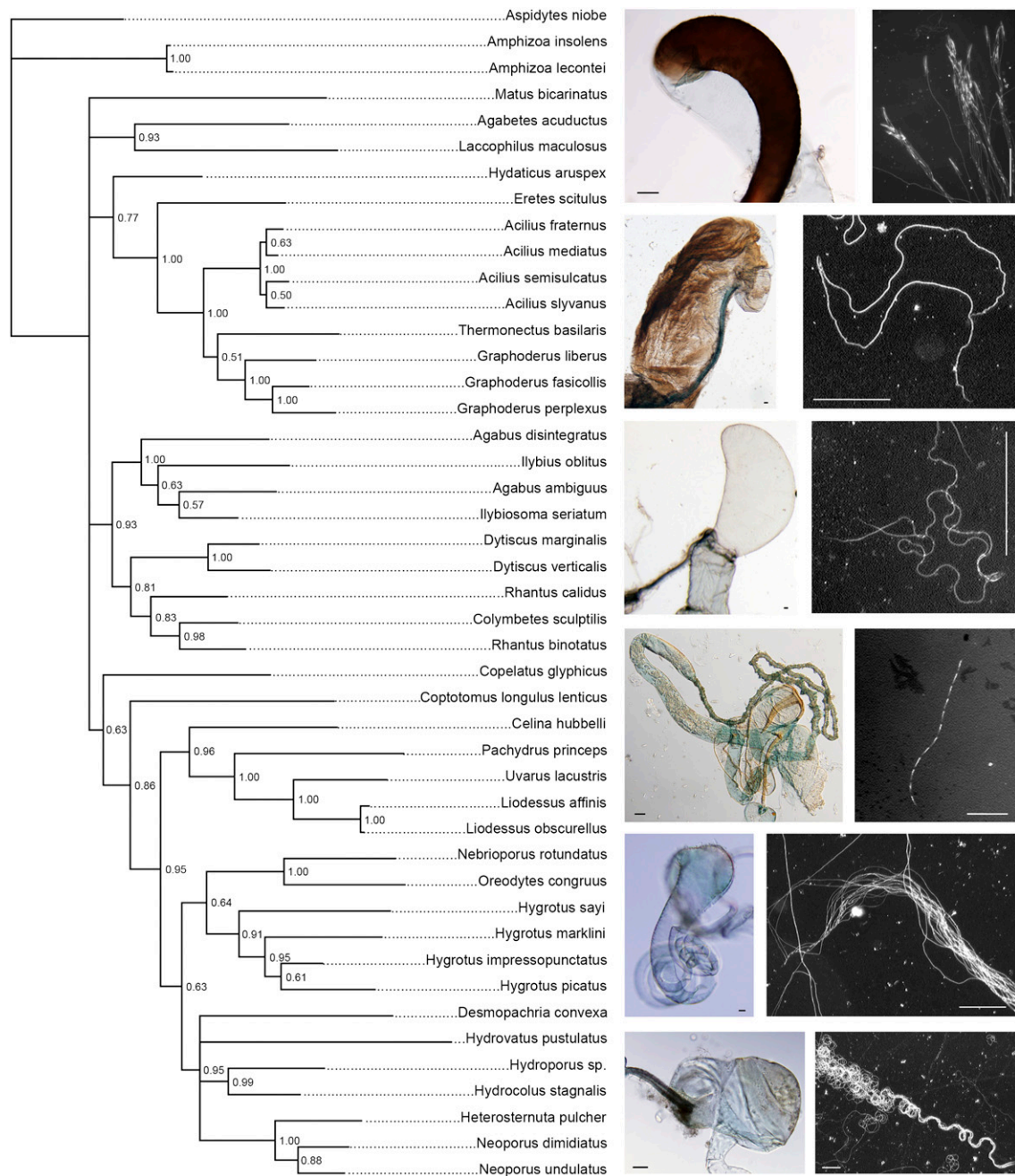
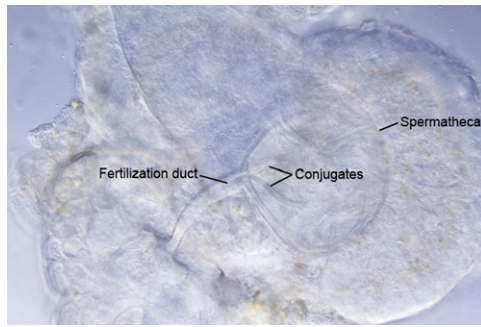


Fig. S1. Majority consensus tree used for phylogenetically controlled generalized least squares and logistic regression analyses. The tree was derived from 20,800 post burn-in trees from four independent Markov chain Monte Carlo runs. Node values indicate the posterior probability of a branching event. Images to the *Right* of the tree illustrate some of the observed diversity of female reproductive tract design and sperm form (*Top to Bottom* row: *Laccophilus maculosus*, *Acilius semisulcatus*, *Rhantus binotatus*, *Uvarus lacustris*, *Hydrovatus pustulatus*, and *Neoporus dimidiatus*). *Acilius* have paired sperm. *Rhantus* produce variable-sized sperm aggregates. *Neoporus*, *Uvarus*, and *Hydrovatus* have rouleaux-type conjugation. Uniquely, sperm are single within the seminal vesicles of *Laccophilus* males but are similar in appearance to rouleaux within the fertilization ducts of females. Female reproductive tracts were imaged using differential interference microscopy. Sperm, with the exception of *Uvarus* (fluorescence microscopy of DNA-stained heads; flagella not visible), were visualized with darkfield microscopy. (Scale bars, 50 μm .)



Movie S1. Motile conjugates within the female reproductive tract of *Neoporus*. The reproductive tract was dissected in supplemented Grace's insect cell culture medium (Invitrogen) and visualized with differential interference microscopy. The spermatheca, fertilization duct, and sperm conjugates are clearly visible. Conjugates are oriented with their tips within the fertilization duct.

[Movie S1](#)

Dataset S1. Mean trait values of female reproductive tract and sperm characters. One to eight individuals were used to calculate means (indicated in parentheses). All measures are in millimeters (or mm^2 for area). Body size was obtained from refs. 1 and 2; *Dytiscus marginalis* sperm characters from refs. 3 and 4

[Dataset S1 \(XLS\)](#)

1. Larson DJ, Alarie Y, Roughley RE (2000) Predaceous diving beetles (Coleoptera: Dytiscidae) of the Nearctic. (NRC Research Press, Ottawa, ON, Canada).
2. Miller KB (2002) Revision of the genus *Eretes* Laporte, 1833 (Coleoptera: Dytiscidae). *Aquatic Insects* 24:247–272.
3. Dallai R, Afzelius BA (1987) Sperm ultrastructure in the water beetles (Insecta, Coleoptera). *Bollettino di Zoologia* 54:301–306.
4. Werner G (1976) Development and structure of the paired sperm of the dytiscids *Acilius sulcatus* L., *Dytiscus marginalis* L. und *Hydaticus transversalis* Pont. (Coleoptera). *Zoomorphologie*, 83:49.87. German.

Dataset S2. GenBank accession numbers for DNA sequence data. * indicates sequence was from a closely related species to the species of interest

[Dataset S2 \(XLS\)](#)