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Large male bias in collection of *Micropterix facetella* Zeller, 1851 (Lepidoptera, Micropterigidae)

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Micropterigidae are a small family of moths containing approximately 160 described species (van Nieukerken et al. 2011), the majority of which are in the type genus *Micropterix* (Kristensen 1998). Adult micropterigids possess mandibulate mouthparts, lacking the proboscis found in the more recently diverging glossatan lineages of Lepidoptera, and many micropterigid larvae are associated with non-angiosperm plants such as liverworts (Imada et al. 2011). For these reasons, extant micropterigids are frequently referred to as primitive moths. Recent phylogenetic analyses (e.g., Bazinet et al. 2016) have shown that the family Micropterigidae is indeed sister to all other Lepidoptera. Adults are usually diurnal and can be observed feeding on pollen or fern spores (Davis and Landry 2012; Kawahara et al. 2018). However, because of their small size, they are collected relatively infrequently.

In April 2017, while attending the 20th European Congress of Lepidopterology in southern Croatia, the authors collected butterflies and small moths near shrubs and oak trees (tentatively identified as *Quercus pubescens* Willd., 1796; Bašić 2013) on a south-facing rocky karst hillside northeast of the town of Podgora (Fig. 1; see below for specifics about location). Upon closer examination, many of the moths perched on the oaks were adult micropterigids (Fig. 2), though none of them were actually observed feeding at the inflorescences. Multiple trips were made to this site over the duration of the seven-day conference, with all collecting done in the afternoon, between the hours of 12:00 PM and 4:00 PM. There was also one attempt to collect at this site in the late morning, shortly after 10:00 AM, but no moths were observed. A total of 145 micropterigid specimens were collected by the authors. All specimens were later identified as *Micropterix facetella* (C. Zeller pers. comm.), which is one of seven micropterigid species known from Croatia (Karsholt 2013). The identification was further confirmed by DNA barcoding of a male specimen (CNCLEP000171989). Interestingly, approximately 80% of these specimens (117/145) were males.

Figure 1. Hillside near Podgora, Croatia, where *M. facetella* specimens were collected.

Figure 2. *Micropterix facetella*, habitus.

The underlying cause for this 4:1 sex ratio remains unclear, though there are multiple plausible explanations. Females may not fly as often as males, in order to preserve energy for creation of progeny. Alternatively, *Micropterix facetella* may be protandrous; this has never been officially demonstrated due to the difficulties of rearing Micropterigidae, but since there are many examples of males emerging before females in other species of Lepidoptera, it is not without precedent (Wiklund and Fagerström 1977). It is also possible that both sexes emerge simultaneously, but exhibit sexually dimorphic flight behavior. If additional collecting had been done in the morning or during twilight hours, a significantly different sex ratio may have been observed.

Observations of congeneric micropterigids indicate that the 4:1 sex ratio in *M. facetella* may be the result of a particular mating strategy. Kozlov and Zvereva (2006) studied adult activity of *Micropterix maschukella* Alphéraky, and observed that females visited host flowers primarily with the intent to feed, whereas males did so primarily with the intent to mate. These males preferred to visit flowers that were already occupied by other *M. maschukella* males, resulting in an observably biased sex ratio not dissimilar to the one encountered at the Croatian oaks. This sort of mating strategy, analogous to lekking, is rare in Lepidoptera, though it has been observed in some species from the families Nymphalidae (Srygley and Penz 1999), Pyralidae (Alem et al. 2011) and Hepialidae (Turner 2015). Similar male aggregations have been observed in other attempts to collect micropterigids (Zeller and Huemer 2015; D. Davis and D. Wagner pers. comm.). Gregarious behavior was also observed in the pollen-feeding *Micropterix calthella* Linnaeus (Erenler and Gillman 2010), though the sex ratio was not recorded. Despite these multiple corroborative studies, the variation in recorded flight time and behavior across all *Micropterix* is significant enough that, with regards to analyzing *M. facetella* behavior, it must all be treated as circumstantial (Zeller-Lukashort et al. 2007)

There are other known occurrences of gender bias in Lepidoptera. The bacterial endosymbiont *Wolbachia* has been found in many lepidopteran families and is known to kill male progeny during early development (Sasaki and Ishikawa 1999; Jiggins et al. 2000; Ahmed et al. 2015, 2016), creating an uneven sex distribution in favor of females. Gender bias towards females has also been observed in gypsy moth adults, due to some parasitoids preferring to parasitize male pupae (Fuester and Taylor 1996). These phenomena are less likely to be causing the 4:1 male-to-female ratio in *M. facetella*, but should not be entirely ruled out.

Although our observations of *M. facetella* are interesting on their own, it will require more than a single week of collecting in order to properly interpret them. Multiple avenues of additional research are needed in order to accurately infer the underlying cause of the observed male bias. Replicating the experimental designs of Kozlov and Zvereva (2006) with *M. facetella* would not only help determine whether the males are lekking, but would also help determine whether the females were actually feeding on oak flowers. This would also enable an assessment of whether the bias is strictly due to variation in diel activity (Kawahara et al. 2018). Rearing *M. facetella* would be necessary to test the protandry hypothesis, though there are relatively few instances of successful rearing of micropterigids from egg to adult (e.g., Carter and Dugdale 1982). Finally, the ethanol-preserved *M. facetella* specimens could be sequenced in order to test for the presence of *Wolbachia* DNA.

Material examined. 110 ♂, 28 ♀. CROATIA: Split-Dalmatia County, nr. Podgora; 43.256°N 17.086°E; 23–30.iv.2017; N.T. Homziak, A.Y. Kawahara, D.M. Plotkin (Molecular collection at the McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, Florida); 7♂, same data, V. Nazari, A.Y. Kawahara (Canadian National Collection of Insects, Ottawa, Ontario, Canada).

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