

# A protocol for sampling, rearing and preserving leaf-mining insects

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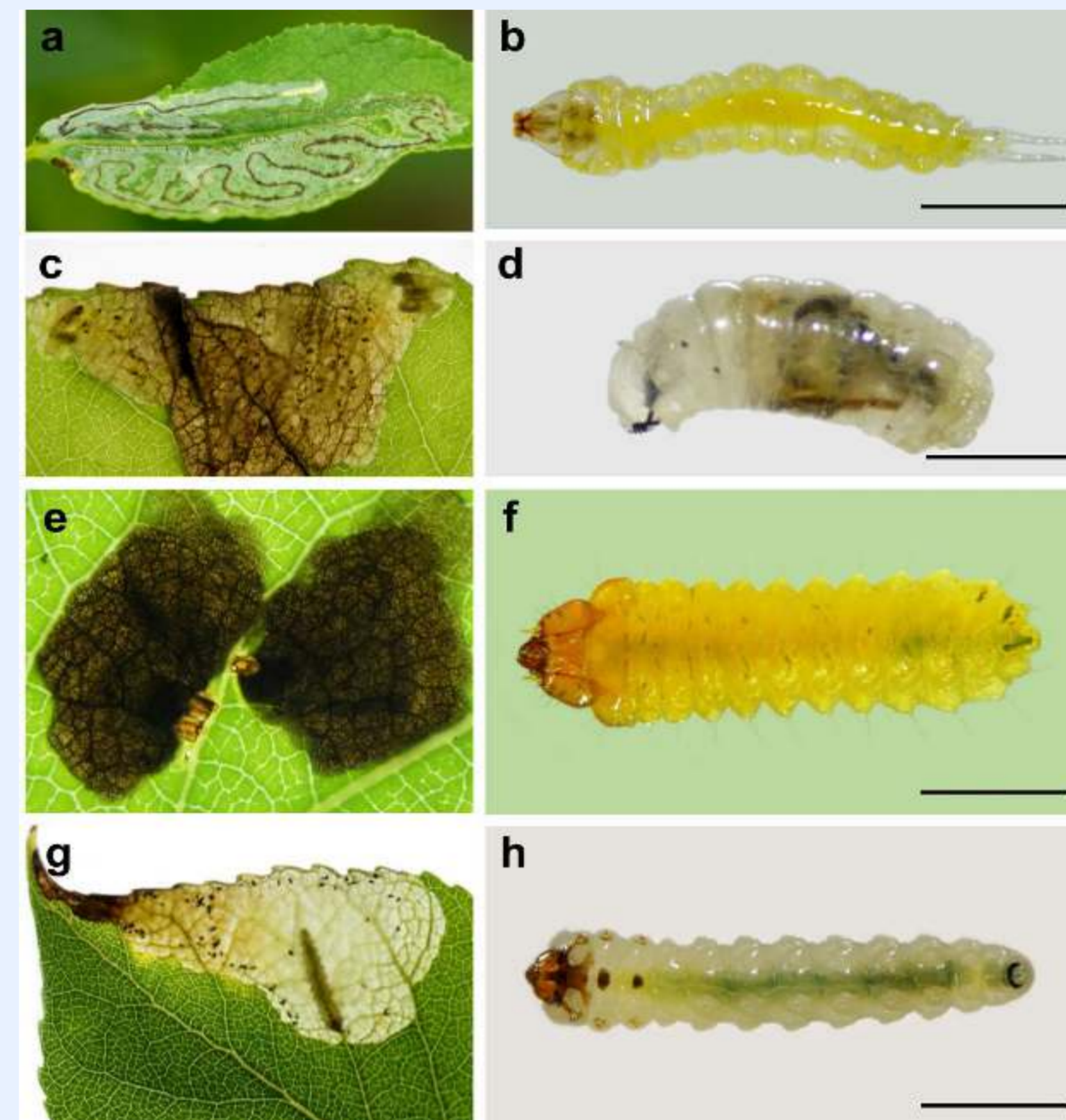
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Leaf miners are a highly diverse group of insects those larvae feed inside leaves making cavities, i.e. leaf mines (Fig. 1).

Many leaf-miners are highly invasive and can cause serious damage to plants. Indeed, some species can lead to losses of different crops in agriculture, others are known as forest and ornamental pests.

Their diversity, trophic associations, ecological and economic importance are still poorly studied, especially in the remote regions of the world.

The goal of the study was to overview the approaches for sampling, rearing and preserving leaf-mining insects, as well as for storing and cataloguing mined leaves, facilitating further studies of leaf-miners.



← Fig. 1. Leaf mines and larvae of leaf-mining insects from four orders found on poplars, *Populus* spp., in Siberia: (a, b) *Phyllocnistis labyrinthella* (Lepidoptera: Gracillariidae); (c, d) *Agromyza albitarsis* (Diptera: Agromyzidae); (e, f) *Zeugophora scutellaris* (Coleoptera: Megalopodidae); (g, h) *Heterarthrus ochropoda* (Hymenoptera: Tenthredinidae). Novosibirsk, Central Siberian botanical garden, 2009–2011 (Lopez-Vaamonde et al., 2020).

## RESULTS

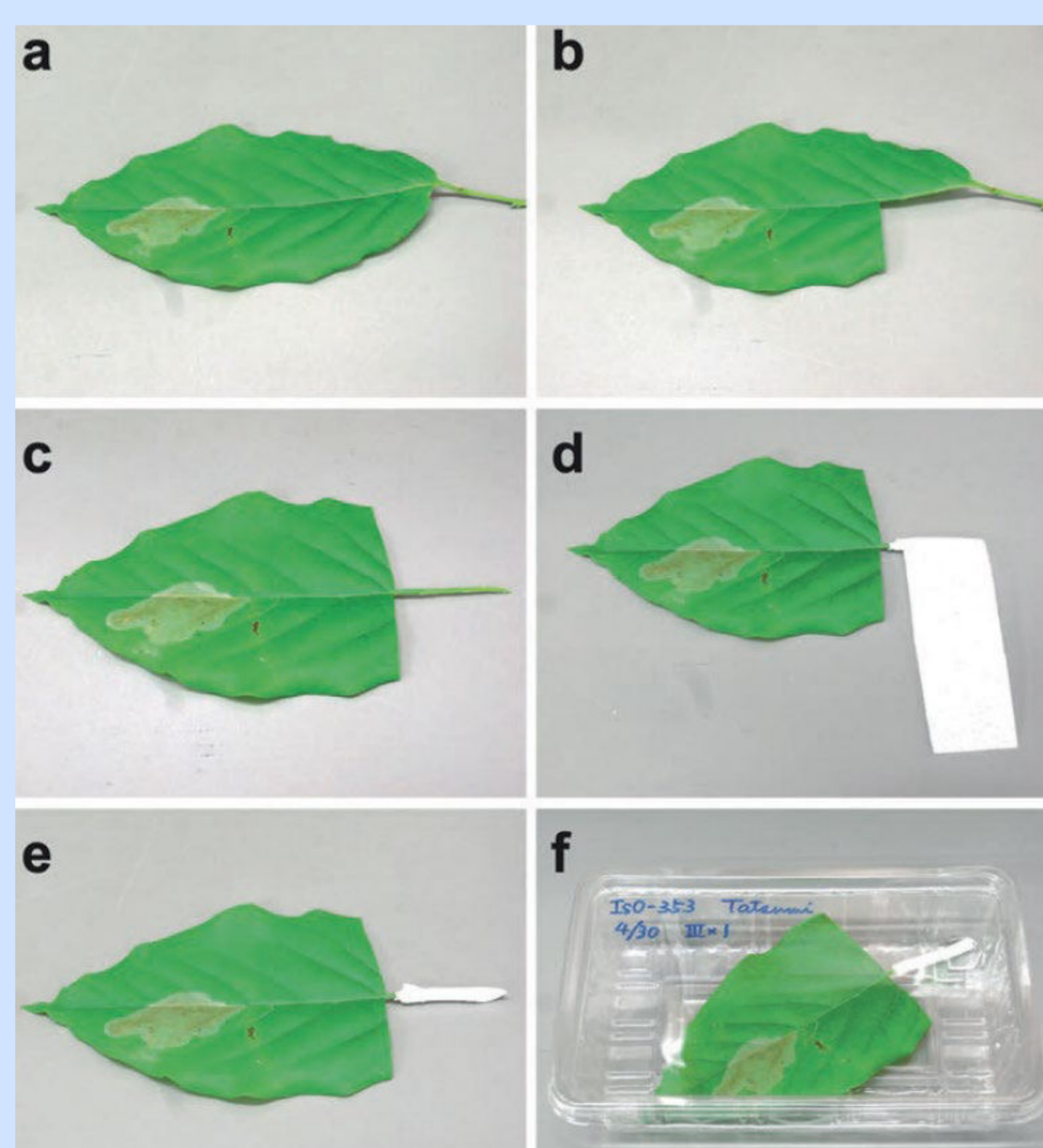
Here we present a protocol for sampling, rearing and preserving leaf miners and their mines with a focus on leaf-mining Microlepidoptera.

### (1) Collecting Leaf Mines

Leaf mines of a target species should be collected from several plant individuals, in a plastic zip-lock bag. Every collecting event is illustrated with photographs of the collected leaves. The field label containing a code is attached to a twig or branch of the sample and should be linked to the leaf-mining insect that emerged from the preserved mines.

### (2) Rearing Leaf-Mining Insects

Mined leaves are to be transferred to rearing boxes individually. Cut spare leaf tissue to give more access to the petiole, wrap the latter and moisturize the petiole with a 2% sucrose solution using a pipette or syringe; place mined leaves into rearing boxes (Fig. 2).



For the leaf-miners that have the ability to exit the initial mines and start secondary mining in new leaves, adding extra new fresh leaves is required.

← Fig. 2. Preparation of mined leaves for rearing: (a) a mined leaf; (b, c) cutting the basal part of the leaf body but leaving the main vein; (d–f) rolling up the left main vein by tissue paper, transferring the leaf to the rearing box and adding sucrose solution (Lopez-Vaamonde et al., 2020).

### (3) Mounting and Preserving Adults

Emerged adults are sampled in small tubes and inactivated by ethyl acetate fumes. Given small size of adults, a stereomicroscope is required to pin them using minute pins. Specimen labeling is crucial.

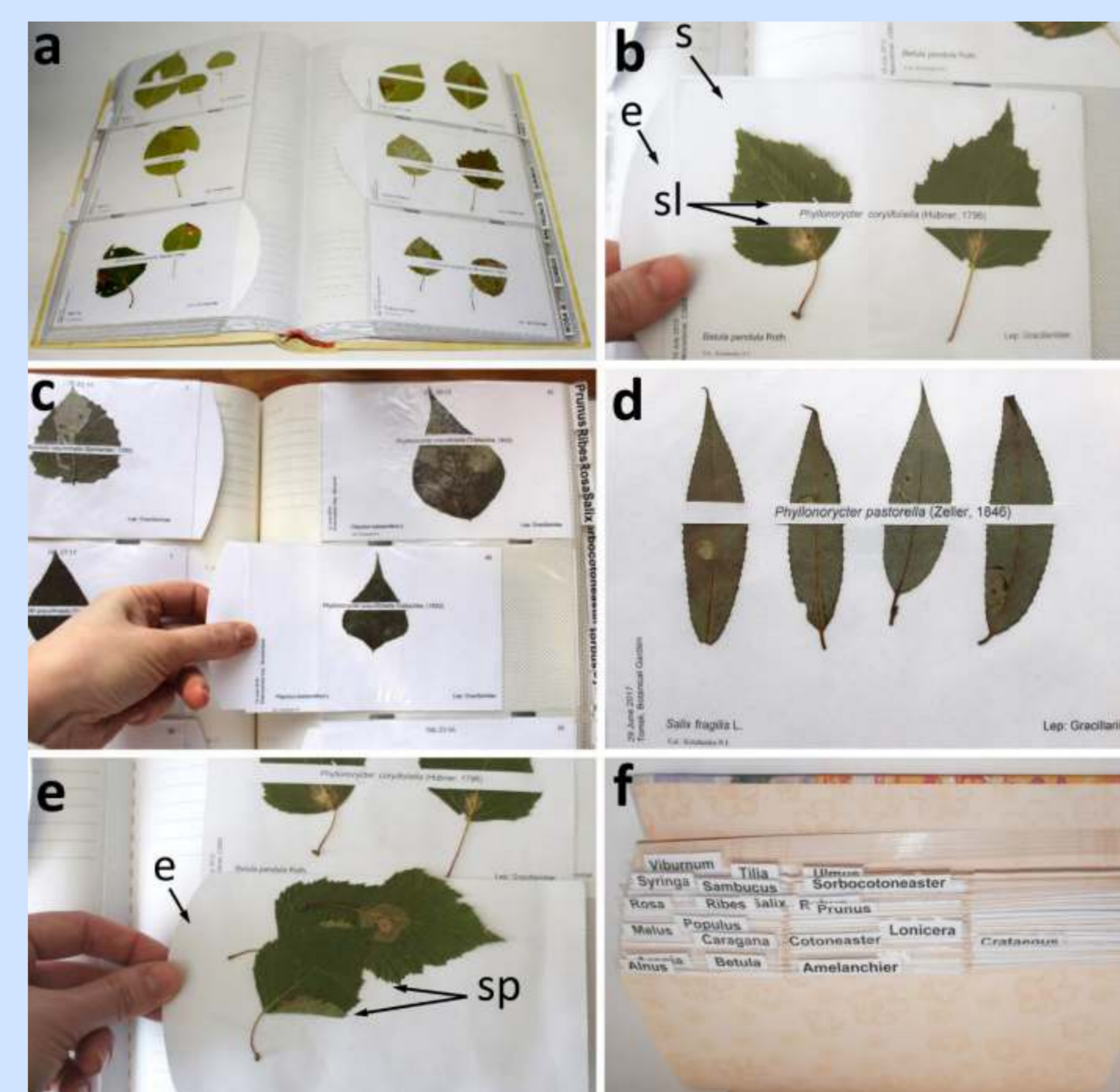
### (4) Fixing and Preserving Immature Stages

It is important to preserve samples of larvae and/or pupae for morphological & molecular analysis, esp. when adult rearing is complex.

Mines are dissected by syringe, larvae and pupae are transfer to tubes with ethanol ≥96% using fine tweezers or a fine brush; tubes are properly labeled and stored in a freezer at –20 °C to low DNA degradation.

### (5) Pressing and Storing Mined Leaves

Mined leaves are dried in newspaper sheets. For compact storing and providing easy access to herbarized specimens, the photo albums (21×33 cm) with transparent pockets can be used (Fig. 3). Dried leaves are fixed on paper supports (10×15 cm), labeled and stored in the album pockets. Duplicates are enveloped & stored under the labeled specimen.



← Fig. 3. Preparation of herbarium: (a) annotated herbarium album with pressed leaves; (b–d) arrangement of pressed leaves on the labeled paper support; (e) paper envelope with duplicates; (f) side labels with the genus name of plants. Arrows: s – labeled paper support with pressed leaves; e – envelope with duplicates; sl – a slot accommodating herbarized leaf; sp – duplicates stored in the envelope (Lopez-Vaamonde et al., 2020).

## READ FOR MORE DETAILS:

Lopez-Vaamonde C., Kirichenko N., Ohshima I. (2020) Collecting, rearing, and preserving leaf-mining insects. Chapter 17. In: J.C. Santos; G.W. Fernandes (eds) Measuring arthropod biodiversity: a handbook of sampling methods. Springer, Cham. pp 439–466. [https://doi.org/10.1007/978-3-030-53226-0\\_17](https://doi.org/10.1007/978-3-030-53226-0_17)

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