

Additional file 3. Parsimony haplotype network analysis.

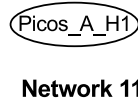
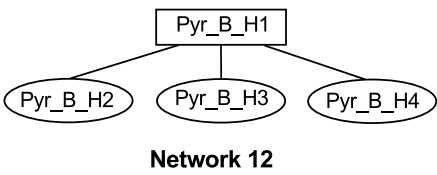
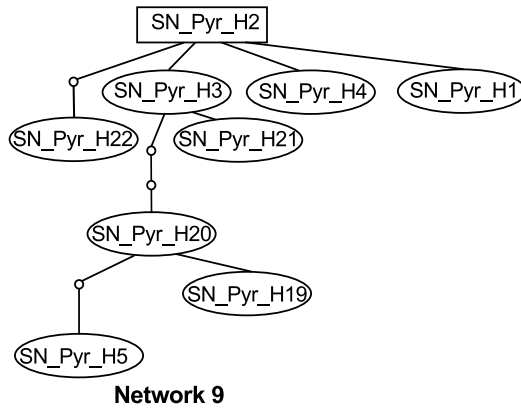
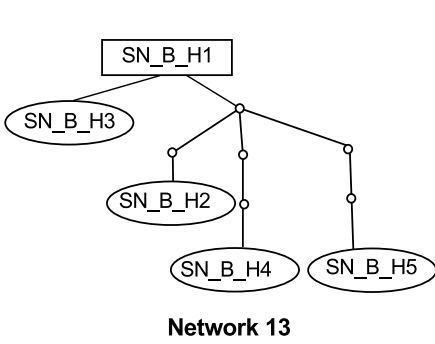
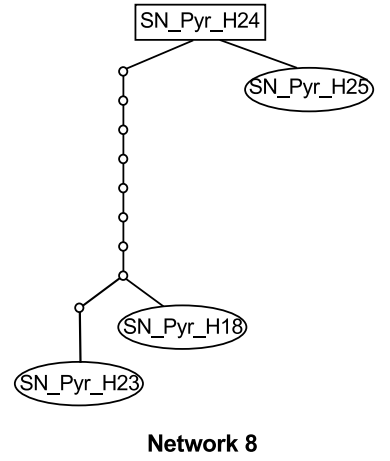
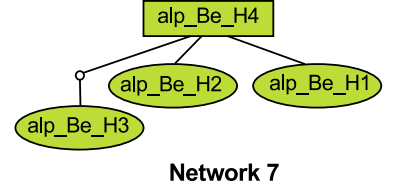
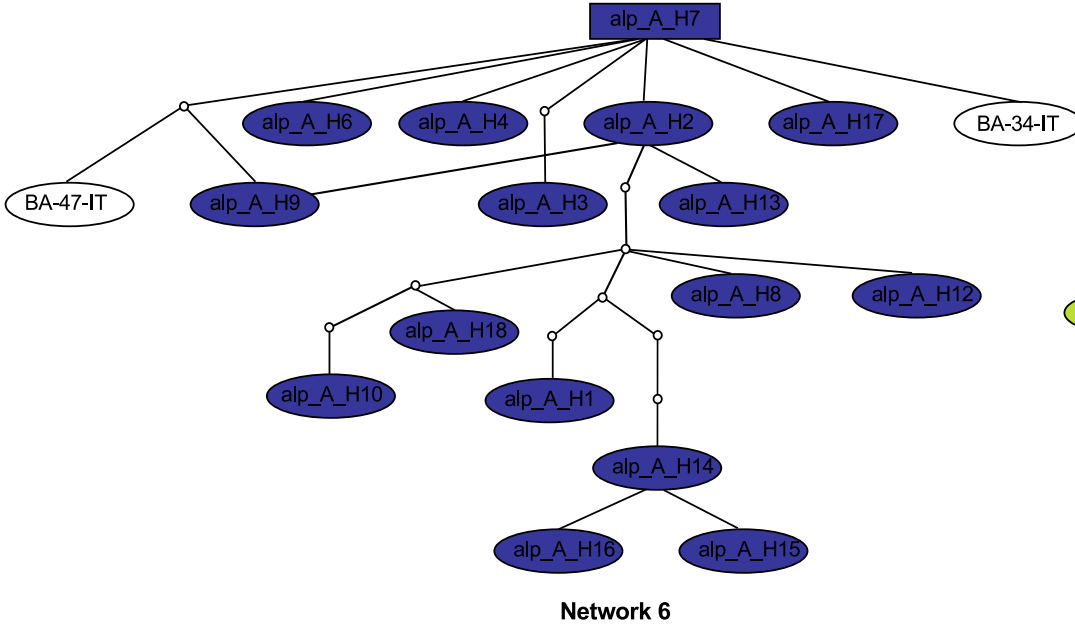
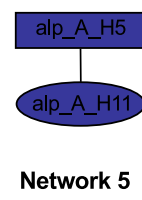
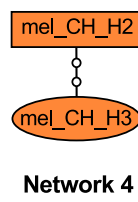
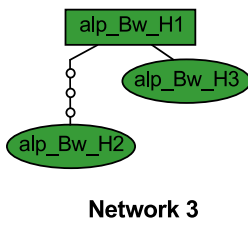
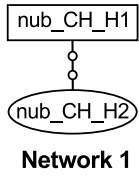
Methods: We performed a Parsimony haplotype network analysis using TCS 1.2 (Clement *et al.* 2000). We tested for species and cryptic lineage delineation by asking whether the COI haplotype data lead to multiple separate networks with the 95% plausible connection limit. Haplotypes separated by more mutational steps (i.e., the probability of secondary mutations exceeds 5%) remain disconnected. This approach does not rely on assumptions of phylogenetic reconstruction methods such as bifurcating relationships and recent analyses show that the 95% parsimony connection limit can provide an additional and simple quantitative standard for phylogenetic species (Monaghan *et al.* 2006; Hart & Sunday 2007).

Results: Statistical parsimony analysis of these 70 unique haplotypes revealed the presence of 16 distinct networks ($L_{95}=10$ steps). In our data, *B. melanonyx* consisted of one haplotype network, and both *B. alpinus* lineage B (matching the nuclear subgroup level divergence, see Figure 5) and lineage A of two further networks. Of the latter, one network included only two A haplotypes. Networks matched all Finn *et al.* GMYC entities (2013;2014) but one, SN_Pyr, split among three networks. Additionally, one network included both *B. nubecularis* haplotypes.

References:

- Clement M, Posada D, Crandall KA: **TCS: a computer program to estimate gene genealogies.** *Molecular Ecology* 2000, **9**(10):1657-1659.
- Finn DS, Khamis K, Milner AM: **Loss of small glaciers will diminish beta diversity in Pyrenean streams at two levels of biological organization.** *Global Ecology and Biogeography* 2013, **22**(1):40-51.
- Finn DS, Zamora-Muñoz C, Múrria C, Sáinz-Bariáin M, Alba-Tercedor J: **Evidence from recently deglaciated mountain ranges that *Baetis alpinus* (Ephemeroptera) could lose significant genetic diversity as alpine glaciers disappear.** *Freshwater Science* 2014, **33**(1):207-216.
- Hart MW, Sunday J: **Things fall apart: biological species form unconnected parsimony networks.** *Biology Letters* 2007, **3**(5):509-512
- Monaghan MT, Balke M, Pons J, Vogler AP: **Beyond barcodes: complex DNA taxonomy of a south pacific island radiation.** *Proceedings of the Royal Society B-Biological Sciences* 2006, **273**(1588):887-893.

Figure: Network analysis based on statistical parsimony (Clement *et al.* 2000) showing the genealogical relationships of the COI haplotypes in cryptic lineages of *B. alpinus*, *B. melanonyx* and *B. nubecularis*. The labels in the circles refer to the COI haplotypes (see Figure 3; Additional file 1, Table S3). The empty dots indicate the presence of missing haplotype intermediates while the connections are based on the set of plausible solutions with a 95% of parsimony probability.



- BA-36-FR
- Network 15**
- BA-33-IT
- Network 16**
- BA-45-IT
- Network 14**

