



## Darwin's Galapagos gourd: providing new insights 175 years after his visit

### ABSTRACT

The year 2010 marks the 175th anniversary of Charles Darwin's visit to the Galapagos Islands. A recent paper by J. C. Briggs, 'Darwin's biogeography' (*Journal of Biogeography*, 2009, 36, 1011–1017), summarizes Darwin's contributions to the field of biogeography, stressing the importance of his natural history specimens. Here, we illustrate how a plant collected by Darwin during his visit to Floreana and not collected since can provide insights into dispersal to oceanic islands as well as extinction of island plants, based on ancient DNA from Darwin's herbarium specimen.

**Keywords** Cucurbitaceae, Darwin's herbarium, extinction, Galapagos, island biogeography, molecular clock, phylogenetics, plant viruses, *Sicyos*.

During his voyage on the *Beagle*, Charles Darwin industriously collected natural history specimens and sent them home from various ports along the way (Briggs, 2009). On his return to Great Britain, he immediately began supervising work on the scientific results of the expedition, employing the help of specialists to record his fossils, fishes, mammals, birds and plants. Among the 209 plants that Darwin collected on the Galapagos Islands and sent to J. D. Hooker was a new species of Cucurbitaceae, *Sicyos villosus* J. D. Hooker (Fig. 1). Unlike his bird collections, Darwin labelled his plants by island, which is why we know that this cucurbit came from Charles Island, now Floreana. On the herbarium label, the species is described as 'In great beds injurious to vegetation.' Since then, this species has never been found again, despite intense search efforts of several botanists. Based on one fruit from a bag attached to Darwin's 175-year-old specimen in the Cambridge

herbarium (Fig. 1, insert), one of us (H.S.) generated nuclear and chloroplast sequences to find out the affinities of this vanished mystery plant. We also tested its relationship to another endemic Galapagos cucurbit, the Santa Cruz gourd, *Sicyocaulis pentagonus* Wiggins, which is known from five collections on Santa Cruz and Isabela, but has not been collected in the past 35 years.

Based on molecular data (Kocyan *et al.*, 2007; Schaefer *et al.*, 2008, 2009), the New World Sicyoeae comprise a clade of about 150 species in 20 genera (*Apatzingania*, *Brandegia*, *Cyclanthera*, *Echinocystis*, *Echinopepon*, *Elateriopsis*, *Frantzia*, *Hanburia*, *Linnaeosicyos*, *Marah*, *Microsechium*, *Parasicyos*, *Pseudocyclanthera*, *Rytidostylis*, *Sechiopsis*, *Sechium*, *Sicyocaulis*, *Sicyos*, *Sicyosperma*, *Vaseyanthus*). Herbarium specimens of 76



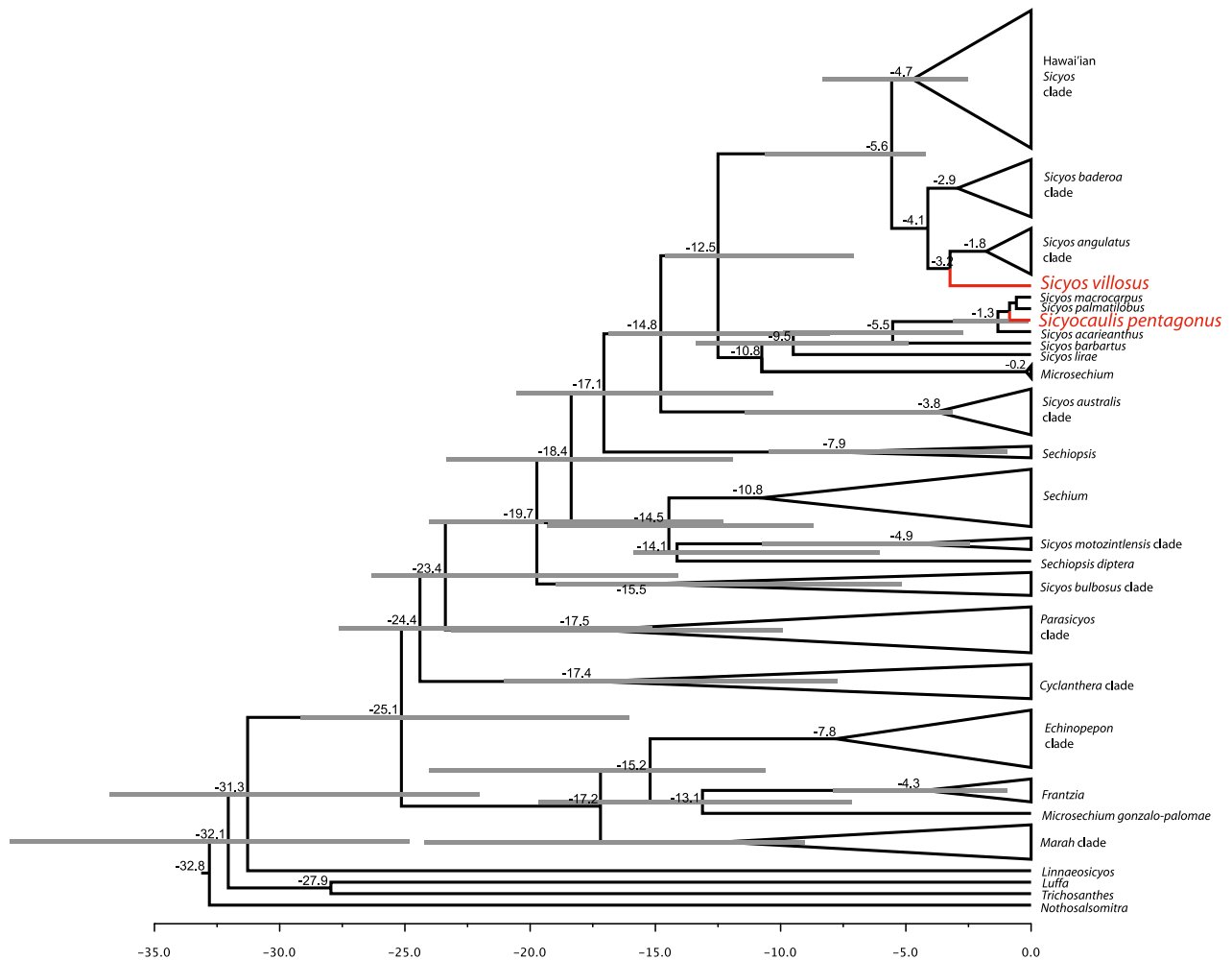
**Figure 1** The sole herbarium collection in existence of *Sicyos villosus*, collected by Darwin during his visit to Floreana in September 1835 and described as a new species by J. D. Hooker. The specimen is now in the Darwin herbarium, whose keeper, Professor J. Parker, granted permission to extract DNA from one of the seeds shown in the inset in the lower right (inset scale bar = 5 mm). Photograph by A. Tye.

species representing all these genera were sequenced for the chloroplast regions *rpl20-rps12* and *trnL/trnL-F*, and the entire nuclear ribosomal RNA intergenic spacer region (ITS1–5.8S–ITS2), following the methods described in Kocyan *et al.* (2007) and Schaefer *et al.* (2008). The genus *Sicyos* was represented with 41 of its *c.* 60 species. Trees were rooted on *Nothoalsomitra* (Schaefer *et al.*, 2009). Herbarium vouchers and GenBank accession numbers are given in the cited papers and in a phylogenetic study of *Sicyos* (P. Sebastian, H. Schaefer, and S. Renner, in preparation). Sequences were edited with SEQUENCHER (v.4.9; Gene Codes, Ann Arbor, MI, USA) and aligned by eye, using MACCLADE v.4.06 (Maddison & Maddison, 2000). The aligned plastid matrix comprised 1818 nucleotides, the aligned ITS matrix comprised 644 nucleotides. Analyses of the

separate plastid and nuclear data partitions produced congruent phylogenetic estimates, and the data were therefore concatenated. The complete alignment is available from P.S. Maximum likelihood (ML) analyses as well as ML bootstrapping relied on RAXML v.7.0.4 (Stamatakis *et al.*, 2008; available at: <http://phylobench.vital-it.ch/raxml-bb/>) and used the GTR + G model.

To obtain absolute ages for the divergences between the Galapagos Islands species and their mainland relatives, we used Bayesian time estimation with an uncorrelated-rates model as implemented in BEAST v.1.4.8 (Drummond & Rambaut, 2007). The alignment used for dating excluded all gapped positions and comprised 2113 nucleotides and 79 species. We again used the GTR + G model with four rate categories. Fossil calibration came from

the pollen *Hexacolpites echinatus* from the Oligocene of Cameroon (Salard-Chebouldaef, 1978; Muller, 1985), which is the oldest known hexacolpate Sicyoeae-type pollen. The most conservative assignment of this pollen is to the split between *Linnaeosicyos* versus the remaining New World Sicyoeae (Schaefer *et al.*, 2009). The Oligocene epoch ranges from 33.9 to 23 Ma, and the stratum containing *Hexacolpites* has not been precisely dated; we used an age of  $28.5 \pm 6$  Ma because an analysis of Cucurbitaceae divergence times using first the upper then the lower boundary of the Oligocene found no significant difference (Schaefer *et al.*, 2009). We also constrained the root of the tree to  $37 \pm 3$  Ma based on Schaefer *et al.* (2009). Markov chain Monte Carlo runs extended for five million generations,



**Figure 2** Chronogram obtained under a relaxed clock model applied to 2113 aligned nucleotides (after exclusion of all gaps) from chloroplast and nuclear DNA sequences from 79 accessions of Sicyoeae (Cucurbitaceae). Darwin's *Sicyos villosus* is closest to species from North America and Mexico, while the Santa Cruz gourd, *Sicyocaulis pentagonus*, is closest to species from Peru and Ecuador; the two lineages also reached the archipelago at different times.

sampling every 1000th generation. Of the 5001 posterior trees, we excluded the first 1000 as burn-in. Convergence was checked using TRACER v.1.4.1 (Rambaut & Drummond, 2007). The estimated covariance parameter was 0.68, and the 95% highest posterior density (HPD) did not enclose 0, justifying the hypothesis of non-autocorrelated rate variation. Results under a strict clock model were similar to those obtained with the relaxed clock model.

A chronogram for 79 species of Sicyoeae–Cucurbitaceae, including 68% of the *c.* 60 species currently assigned to *Sicyos*, shows that both Galapagos cucurbits belong to this widespread genus, which has also diversified in the Hawaiian archipelago and in Australia and New Zealand (Fig. 2). Darwin's *Sicyos villosus* is closest to species from North America and Mexico, while the Santa Cruz gourd, *Sicyocaulis pentagonus*, is closest to species from Peru and Ecuador. The molecular clock suggests that the divergence from their respective closest relatives occurred 4 ( $\pm$  2) Ma in *Sicyos villosus*, and 1.4 ( $\pm$  1.2) Ma in *Sicyocaulis pentagonus*. Thus, the two species arrived on the Galapagos archipelago through non-anthropogenic long-distance dispersal from different continental source populations and at times that match the geological age of the islands: the Galapagos Islands are the product of hotspot activity 930 km west of the Ecuadorian coast and are at least 3–4 Myr old (Hickman & Lipps, 1985). Most plant species (up to 60%) appear to have arrived via birds, and the closest floristic ties are with Ecuador and Peru, followed by Central America and Mexico (Porter, 1976). It is also known that storm petrels migrate between Peru and the Galapagos (Tomkins, 1982) and that these and other seabirds nest in habitats where *Sicyos* occurs (Marks, 1992), suggesting that the spiny fruits (Fig. 1) may have been carried by birds.

Floreana, where Darwin collected *Sicyos villosus*, was settled in 1807 and continuously inhabited with only short breaks of a few years' abandonment during the 19th century. The settlers brought livestock, and by the time Darwin visited (in 1835) they owned approximately 2000 head of cattle (Steadman, 1986). Markham, who visited Floreana in 1880, found it 'in undisturbed possession of the so-called wild cattle ... donkeys, dogs, pigs, and other animals that had been left to run wild on the abandonment of the island by the former inhabitants' (Steadman, 1986, p. 62). A plausible explanation for the disap-

pearance of Darwin's gourd could be that it was grazed to extinction. And yet many Galapagos plants may be adapted to grazing: prior to the arrival of feral animals some 2–3 million giant tortoises (*Geochelone nigra*) lived on the archipelago and fed on its vegetation (Fowler de Neira, 1985; Coblenz & Baber, 1987). Tortoises, however, are inefficient grazers compared with goats and cattle, and the reach of the animals is different. There are many examples of Galapagos plants being apparently or obviously grazed to extinction or extreme rarity (A. Tye, personal communication, 18 January 2010). However, the speed of the disappearance of a plant described as abundant in 1835 is surprising.

Another plausible cause for the decline of *Sicyos villosus* (and possibly also *Sicyocaulis pentagonus*) could be Cucurbitaceae-specific viruses (e.g. cucumber mosaic virus, watermelon mosaic virus, zucchini yellow mosaic virus) introduced with cucurbit crops cultivated by the settlers. The dramatic decline of *Sicyos australis* in New Zealand, a species now almost entirely restricted to small offshore islands where no cucurbit crops are grown, has been attributed at least partly to these viruses (Delmiglio & Pearson, 2006). Similar inadvertent introduction of cucumber viruses could have occurred on the Galapagos Islands. Finally, it is possible that the extinction of Darwin's Galapagos gourd is only apparent and due to infrequent and uneven botanical collecting. In the course of this project, we contacted four botanists who have resided on the Galapagos Islands and made systematic collections there (Henning Adersen, Ole Hamann, Henk van der Werff and Alan Tye). All of them stressed that there are still some areas that have never been subject to a full botanical survey.

As illustrated by the sole specimen in existence of *Sicyos villosus*, Darwin's unique natural history collections continue to shed light on the origin and destruction of the Galapagos biota even 175 years after his visit. And although Darwin himself did not comment on the likely mode of transport of *Sicyos* seeds or fruits, he was acutely aware of the role of birds in plant dispersal (Briggs, 2009) and may well have collected the spiny seeds of *Sicyos villosus* (Fig. 1, inset) with this hypothesis in mind.

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## Lessepsian fish migration: genetic bottlenecks and parasitological evidence

### ABSTRACT

As a rule, non-indigenous species (NIS) populations derived from biological invasion events represent a subset of the genetic diversity of the source population. In biological invasions, host–parasite interactions play an important role, and parasitological data for NIS populations can provide useful information such as their area of origin, mechanism of invasion and prospects of success in the new habitat. When both genetic and parasitological data are available, and they suggest the same scenario, the history of an invasion can be inferred with no discrepancy, but when data cannot be reconciled an alternative model should be considered. In this study a comparison of genetic and parasitological data for the Lessepsian migrant the bluespotted cornetfish, *Fistularia commersonii*, in the Mediterranean Sea presents the opportunity to evaluate the compatibility of information of this nature, and to propose possible

invasion scenarios consistent with evidence provided by both criteria.

**Keywords** Biological invasions, fish migration, *Fistularia commersonii*, genetics, Lessepsian migrations, Mediterranean Sea, parasitology.

### GENETIC AND PARASITOLOGICAL ASPECTS OF SPECIES INVASIONS

Genetic data can be useful for elucidating the history and phenomena involved in species invasions. In recent years, it has increasingly been emphasized that many natural populations that derived from a founder event and/or a bottleneck represent a subset of the genetic diversity of the source population, leading to the conclusion that the genetic diversity of the newly founded populations should be significantly lower than that of the source (Tsutsui *et al.*, 2000; Moum & Arnason, 2001; Sax *et al.*, 2005). Conversely, other types of evidence could also be used to retrace the route taken by invasive species. For instance, parasitological data might provide useful information about the area of origin of invaders, but might also shed light on some further aspects such as the mechanism of invasion (e.g. whether they arrived as adults or in the larval stages) or the prospects of success in the new habitat (e.g. potential infection with native and/or natural parasites) (Diamant, 1989; Poulin & Mouillot, 2003; Galli *et al.*, 2007; Merella *et al.*, 2007; Pais *et al.*, 2007, 2008).

When both genetic and parasitological data are available, and suggest the same invasion scenario, the history of a particular invasion can be inferred with a certain degree of reliability. However, when these data on the same invasive species cannot be reconciled because they suggest a different route or history – assuming that both genetic and parasitological evidence are equally valid – an alternative model should be considered.

In this study a comparison of genetic and parasitological data for a Lessepsian migrant fish species presents the opportunity to suggest a multiple-approach method for a better understanding of the events that led to the invasion of the new range. In fact, the complementarity of such information greatly strengthens likely invasion scenarios suggested by both types of criteria.

### LESSEPSIAN FISH MIGRANTS AND GENETIC DIVERSITY

Since the opening of the Suez Canal in 1869, the Mediterranean Sea has been subject to an invasion of Indo-Pacific species from the Red Sea (Por, 1971). As far as fishes are concerned, it has recently been estimated that the so-called Lessepsian migrants include more than 60 species (Ben Rais Lasram *et al.*, 2008). Contrary to the evidence of a decrease in genetic diversity of invader species, analyses carried out to date on Lessepsian fish species reveal no significant reduction in genetic diversity, that is, conspecific populations from the Red Sea–Indo-Pacific region and the Mediterranean basin are genetically similar (Bucciarelli *et al.*, 2002; Hassan *et al.*, 2003; Hassan & Bonhomme, 2005). A likely explanation is that Lessepsian fish species invaded the Mediterranean with a conspicuous number of individuals, either as adults or larval stages, which reduced the loss of genetic diversity.

### THE CASE OF *FISTULARIA COMMERSONII*

The bluespotted cornetfish, *Fistularia commersonii* Rüppell (Osteichthyes: Fistulariidae), is an Indo-Pacific species whose presence was recorded for the first time on the Mediterranean coast of the Middle East (Golani, 2000). Later, its geographical distribution rapidly extended to the eastern basin (Corsini *et al.*, 2002), and more recently to the central and western Mediterranean (Pais *et al.*, 2007; Dulčić *et al.*, 2008). Knowledge of the specific dynamics of the Mediterranean invasion by *F. commersonii* is meagre. Golani *et al.* (2007), studying the partial sequence of the mitochondrial (mt) DNA control region (D-loop 1, primers in Lee *et al.*, 1995), suggest the occurrence of a genetic bottleneck in the Lessepsian migration of this species. These authors believe that the very low richness and diversity of the haplotypes found in the Mediterranean Sea (52 individuals, 2 haplotypes, haplotype diversity 0.009), compared with those from the natural range (49 individuals, 46 haplotypes, haplotype diversity 0.997), indicate that ‘...the Mediterranean populations of bluespotted cornetfish represent a single invasion event by as few as two females’ (Golani *et al.*, 2007, p. 544). The same authors attribute the rapid expansion of this fish in the Mediterranean Sea to larval dispersal favoured by water circulation.