



## Revision of *Hygrochilus* (Orchidaceae: Epidendroideae: Aeridinae) and a molecular phylogenetic analysis

MING-HE LI<sup>1</sup>, GUO-QIANG ZHANG<sup>2</sup>, ZHONG-JIAN LIU<sup>2</sup> & SI-REN LAN<sup>1</sup>

<sup>1</sup>College of Forestry, Fujian Agriculture and Forestry University, Fuzhou 350002, China; E-mail (S.-R. Lan): lkzx@safu.edu.cn

<sup>2</sup>Shenzhen Key Laboratory for Orchid Conservation and Utilization, The National Orchid Conservation Center of China and The Orchid Conservation & Research Center of Shenzhen, Shenzhen 518114, China

### Abstract

*Hygrochilus* and *Sedirea* are genera of orchids with only three species endemic to Asia. An analysis of ITS and five plastid regions using parsimony, maximum likelihood, and Bayesian methods obtain clear evidence that *Sedirea* is nested within and should be considered synonymous with *Hygrochilus*. We adopt a broadly defined *Hygrochilus* characterized by possession of four pollinia. A new combination, namely, *Hygrochilus japonica*, and a new species, namely, *Hygrochilus tsii* (Orchidaceae: Epidendroideae: Aeridinae), are proposed.

**Key words:** *Aerides*, Asian orchids, orchid phylogenetics, *Ornithochilus*, *Phalaenopsis*, *Sedirea*, Vandeae

### Introduction

Aeridinae (Orchidaceae) are characterized by monopodial growth with highly developed velamen, and numerous genera in this subtribe possess a column foot and a spurred lip (Hidayat *et al.* 2005, Hidayat 2006, Stpiczyńska *et al.* 2011). Understanding relationships within Aeridinae is difficult because of morphological diversification and possible parallelism of vegetative and reproductive features (Kocyan *et al.* 2008). Although molecular analyses (Hidayat *et al.* 2005, Kocyan *et al.* 2008, Gardiner *et al.* 2013) have been performed and pollinaria morphology (Hidayat 2006) and nectary spur anatomy (Stpiczyńska *et al.* 2011) of Aeridinae have been studied, positions of a few genera, e.g., *Hygrochilus* Pfitzer in Engler & Prantl (1897: 112) and *Sedirea* Garay & Sweet (1974: 149), are unclear.

*Hygrochilus* was based on *Vanda parishii* Reichenbach (1868: 138) due to the complete division of each pollinium into two unequal halves, and it has been recently maintained as distinct by most orchidologists (Hidayat *et al.* 2005, Chen *et al.* 2009). It was monotypic for most of its history and endemic to India, Laos, Myanmar, Thailand, Vietnam, and Yunnan Province of China (Chen *et al.* 2009). Previously, *Hygrochilus parishii* (Rchb.f.) Pfitzer in Engler & Prantl (1897: 112) was the only species recognized in the genus until Tsi published *H. subparishii* Tsi (1982: 267), which is distributed from central to southern China (Chen *et al.* 2009). However, Christenson (1985) transferred *H. subparishii* to *Sedirea* based on differences between these two species, which differ in terms habit and column and spur length, resulting in *Hygrochilus* again being monotypic.

*Sedirea* is a controversial genus originally based upon *Aerides japonica* Reichenbach (1863: 210), which differs from *Aerides* Loureira (1790: 525) in its long column and short column foot. *Sedirea* had been monotypic and endemic to Japan, Korea and Yunnan and Zhejiang Provinces of China, but not to any other parts of China (Chen & Tsi 1992, Chen *et al.* 2009) until Christenson (1985) transferred *Hygrochilus subparishii* to it. Hidayat *et al.* (2005) suggested that the number of pollinia does not reflect major relationships within Aeridinae, but it might be useful at the generic level. *Sedirea japonica* (Rchb.f.) Garay & Sweet (1974: 149) and *Hygrochilus subparishii* were described as having two pollinia (Reichenbach 1868, Tsi 1982, Hidayat 2006, Chen *et al.* 2009). *Hygrochilus* and *Sedirea* are morphologically similar, but these hypotheses still need to be evaluated using molecular data.

## New combination

*Hygrochilus japonica* (Rchb.f.) M.H.Li, Z.J.Liu & S.R.Lan, *comb. nov.* (萼脊湿唇兰); basionym *Aerides japonica* Reichenbach (1863: 210).  
Homotypic synonym: *Sedirea japonica* (Rchb.f.) Garay & Sweet (1974: 149).

## Acknowledgements

This study is supported by grants from the Science and Technology Research and Development Funds of Shenzhen, China (No. JC201005310689A). We are grateful to Peiwen Zhang for the illustration, to Liu-Yin Chen and Long-Hai Zou for the analysis of molecular sequence data and to Li-Jun Chen and Wen-Hui Rao for the field support and map elaboration.

## References

- Bentham, G. & Hooker, J.D. (1883) *Genera plantarum*. Reeve, London, 581 pp.
- Chase, M.W. (1987) Systematic implications of pollinarium morphology in *Oncidium* Sw., *Odontoglossum* Kunth and allied genera (Orchidaceae). *Lindleyana* 2: 8–28.
- Chen, S.C. & Tsi, Z.H. (1992) On some imperfectly known genera in Chinese orchid flora. *Acta Phytotaxonomica Sinica* 30: 256–262.
- Chen, S.C., Liu, Z.J., Zhu, G.H., Lang, K.Y., Ji, Z.H., Luo, Y.B., Jin, X.H., Cribb, P.J., Wood, J.J. & Gale, S.W. (2009) *Flora of China: Orchidaceae*. Science Press, Beijing, 382 pp.
- Christenson, E.A. (1985) The generic reassignment of *Hygrochilus subparishii* Tsi (Orchidaceae: Sarcanthinae). *Taxon* 34: 516–518.  
<http://dx.doi.org/10.2307/1221229>
- Demesure, B., Sodzi, N. & Petit, R.J. (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* 4:129–131.  
<http://dx.doi.org/10.1111/j.1365-294x.1995.tb00201.x>
- Engler, A. & Prantl, K.A.E. (1897) *Die natürlichen Pflanzenfamilien*. Engelmann, Leipzig, 112 pp.
- Freudenstein, J.V. & Rasmussen, F.N. (1996) Pollinium development and number in the Orchidaceae. *American Journal of Botany* 83: 813–824.  
<http://dx.doi.org/10.2307/2446258>
- Garay, L.A. & Sweet, H.R. (1974) *Orchids of Southern Ryukyu Islands*. Harvard University, Cambridge, 149 pp.
- Gardiner, L.M., Kocyan, A., Motes, M., Roberts D.L. & Emerson, B.C. (2013) Molecular phylogenetics of *Vanda* and related genera (Orchidaceae). *Botanical Journal of the Linnean Society* 173: 549–572.  
<http://dx.doi.org/10.1111/boj.12102>
- Hidayat, T., Yukawa, T. & Ito, M. (2005) Molecular phylogenetics of subtribe Aeridinae (Orchidaceae): insights from plastid *matK* and nuclear ribosomal ITS sequences. *Journal of Plant Research* 118: 271–284.  
<http://dx.doi.org/10.1007/s10265-005-0217-3>
- Hidayat, T. (2006) Evolutionary analysis of pollinaria morphology of subtribe Aeridinae (Orchidaceae). *Reinwardtia* 12: 223–235.
- Kocyan, A., Vogel, E.F.D., Conti, E. & Gravendeel, B. (2008) Molecular phylogeny of *Aerides* (Orchidaceae) based on one nuclear and two plastid markers: A step forward in understanding the evolution of the Aeridinae. *Molecular Phylogenetics and Evolution* 48: 422–443.  
<http://dx.doi.org/10.1016/j.ympev.2008.02.017>
- Lindley, J. (1833) *The genera and species of orchidaceous plants*, Ridgways, London, 242 pp.  
<http://dx.doi.org/10.5962/bhl.title.499>
- Liu, Z.J., Chen, L.J., Chen, S.C., Cai, J., Tsai, W.C., Hsiao, Y.Y., Rao, W.H., Ma, X.Y. & Zhang, G.Q. (2011) *Paraholcoglossum* and *Tsiorchis*, two new orchid genera established by molecular and morphological analyses of the *Holcoglossum* alliance. *PloS One* 6: e24864.  
<http://dx.doi.org/10.1371/journal.pone.0024864>
- Loureiro, J.D. (1790) *Flora cochinchinensis*. Academicis, Lisbon, 525 pp.
- Mike, T., Lena, S. & Joachim, W.K. (1999) The phylogenetic relationships and evolution of the Canarian laurel forest endemic *Ixanthus viscosus* (Aiton) Griseb. (Gentianaceae): evidence from *matK* and ITS sequences, and floral morphology and anatomy. *Plant Systematics and Evolution* 218: 299–317.  
<http://dx.doi.org/10.1007/bf01089233>
- Pridgeon, A.M., Cribb, P.J., Chase, M.W. & Rasmussen, F.N. (2014, in press) *Genera orchidacearum*, Volume 6, Oxford

- University Press, Oxford.
- Reichenbach, H.G. (1868) *Xenia orchidacea*. Brockhaus, Leipzig, 138 pp.
- Reichenbach, H.G. (1863) Aerides Arten. *Hamburger Garten-und Blumenzeitung* 19: 210.
- Rolfe, R.A. (1919) *Staurospis parishii* & *S. mariottiana*. *The Orchid Review* 27: 97.
- Sang, T., Crawford, D.J. & Stuessy, T.F. (1997) Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84: 1120–1136.  
<http://dx.doi.org/10.2307/2446155>
- Schlechter (1912) Orchidaceae novae et criticae. *Repertorium specierum novarum regni vegetabilis*. 11: 47.  
<http://dx.doi.org/10.1002/fedr.4870110109>
- Senghas, K. (1988) Eine neue gliederung der subtribus Aeridinae (=Sarcinthinae). *Orchidee* 39: 219–223.
- Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* 94: 275–288.  
<http://dx.doi.org/10.3732/ajb.94.3.275>
- Stpiczyńska, M., Davies, K.L. & Kamińska, M. (2011) Comparative anatomy of the nectary spur in selected species of Aeridinae (Orchidaceae). *Annals of Botany* 107: 327–345.
- Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.  
<http://dx.doi.org/10.1007/bf00037152>
- Tsi, Z.H. (1982) Two new species of Orchidaceae from China. *Acta Botanica Yunnanica* 4: 267–270.
- Yukawa, T., Miyoshi, K. & Yokohama, J. (2002) Molecular phylogeny and character evolution of *Cymbidium* (Orchidaceae). *Bulletin of the National Science Museum, Tokyo* 28: 129–139.
- Zhang, G.Q., Liu, K.W., Chen, L.J., Xiao, X.J., Zhai, J.W., Li, L.Q., Cai, J., Hsiao, Y.Y., Rao, W.H., Huang, J., Ma, X.Y., Chung, S.W., Huang, L.Q., Tsai, W.C. & Liu, Z.J. (2013) A new molecular phylogeny and a new genus, *Pendulorchis*, of the Aerides–Vanda alliance (Orchidaceae: Epidendroideae). *PloS One* 8: e60097.  
<http://dx.doi.org/10.1371/journal.pone.0060097>