

# **Rapid identification of the invasive fall armyworm *Spodoptera frugiperda* (Lepidoptera, Noctuidae) using species-specific primers in multiplex PCR**

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**Supplementary Table S1.** Information on voucher ID and DNA concentration of each sample used in this study

| <b>Taxon</b>                | <b>Voucher ID</b> | <b>DNA concentration (ng/μl)</b> |
|-----------------------------|-------------------|----------------------------------|
| <i>Euproctis taiwana</i>    | Lep76-1           | -                                |
|                             | Lep80-1           | 3.7                              |
| <i>Helicoverpa armigera</i> | Lep66-1           | 47.2                             |
|                             | Lep495-1          | 41.7                             |
| <i>Hydrillodes lentalis</i> | Lep580-3          | 96.3                             |
| <i>Mythimna separata</i>    | Lep582-1          | 83.6                             |
| <i>Mythimna loreyi</i>      | Lep582-2          | 73.1                             |
|                             | Lep582-3          | 173.3                            |
| <i>Ostrinia furnacalis</i>  | Lep87-1           | 66.1                             |
|                             | Lep87-2           | 18.1                             |
|                             | Lep588-1          | 63.8                             |
|                             | Lep588-2          | 3.2                              |
| <i>Simplicia cornicalis</i> | Lep588-3          | 23.2                             |
|                             | Lep580-1          | 4.0                              |
|                             | Lep580-2          | 4.4                              |
|                             | Lep580-4          | 71.7                             |
| <i>Spoladea recurvalis</i>  | Lep74-1           | 18.2                             |
|                             | Lep89-1           | -                                |
| <i>S. frugiperda</i>        | Lep581-2          | 54.8                             |
|                             | Lep581-3          | 218.6                            |
|                             | Lep581-4          | 25.8                             |
|                             | Lep581-5          | 24.6                             |
|                             | Lep581-6          | 11.6                             |
|                             | Lep581-7          | 23.2                             |
|                             | Lep581-8          | 32.2                             |
|                             | Lep581-9          | 26                               |
|                             | Lep581-10         | 78.2                             |
|                             | Lep581-11         | 65.4                             |
|                             | Lep581-12         | 86.1                             |
|                             | Lep581-13         | 147.6                            |
|                             | Lep581-14         | 19.8                             |
|                             | Lep581-15         | 52.8                             |
|                             | Lep581-16         | 50                               |
|                             | Lep581-17         | 47.9                             |
|                             | Lep581-18         | 101.8                            |
|                             | Lep581-19         | 48                               |
|                             | Lep581-20         | 90.4                             |
|                             | Lep582-4          | 110.3                            |
|                             | Lep583-1          | 85.7                             |
|                             | Lep583-2          | 91.2                             |
|                             | Lep583-3          | 89.5                             |
|                             | Lep583-4          | 26.8                             |
|                             | Lep584-1          | 57.1                             |
|                             | Lep584-2          | 56.2                             |
|                             | Lep584-3          | 102.9                            |
|                             | Lep584-4          | 87.6                             |
|                             | Lep589-1          | 157.2                            |
|                             | Lep590-1          | 120.4                            |
|                             | Lep590-2          | 68.6                             |
|                             | Lep591-1          | 61                               |
|                             | Lep591-2          | 79.9                             |
|                             | Lep592-1          | 62.7                             |
|                             | Lep592-2          | 68.3                             |
|                             | Lep592-3          | 113.1                            |
|                             | Lep592-4          | 82.5                             |
|                             | Lep592-5          | 61.5                             |
|                             | Lep652-1          | 90.4                             |
|                             | Lep652-2          | 117.1                            |
|                             | Lep670-1          | 30.6                             |
|                             | Lep670-2          | 65.9                             |
|                             | Lep670-3          | 62.5                             |
|                             | Lep682-1          | 15.3                             |
|                             | Lep683-1          | 129.9                            |
|                             | Lep683-2          | 105                              |
|                             | Lep683-3          | 171.6                            |
|                             | Lep683-4          | 31.6                             |
|                             | Lep683-5          | 100.3                            |
|                             | Lep683-6          | 140.8                            |
|                             | Lep752-1          | 135.8                            |
|                             | Lep752-2          | 141.8                            |
|                             | Lep752-3          | 62.1                             |
|                             | Lep752-4          | 57.6                             |
|                             | Lep752-5          | 62.7                             |

**Supplementary Table S1 (continued)**

| <b>Taxon</b>         | <b>Voucher ID</b> | <b>DNA concentration (ng/μl)</b> |
|----------------------|-------------------|----------------------------------|
| <i>S. frugiperda</i> | Lep752-6          | 45.9                             |
|                      | Lep752-7          | 31.6                             |
|                      | Lep752-8          | 21.5                             |
|                      | Lep752-9          | 26                               |
|                      | Lep752-10         | 34.4                             |
|                      | Lep752-11         | 43                               |
|                      | Lep752-12         | 25.8                             |
|                      | Lep752-13         | 45.9                             |
|                      | Lep752-14         | 58.6                             |
|                      | Lep752-15         | 21.4                             |
|                      | Lep752-16         | 22.8                             |
|                      | Lep752-17         | 46.2                             |
|                      | Lep752-18         | 24.2                             |
|                      | Lep752-19         | 52.3                             |
| <i>S. litura</i>     | Lep65-1           | -                                |
|                      | Lep65-2           | 72.8                             |
|                      | Lep191-1          | 69.1                             |
|                      | Lep528-1          | 96.5                             |
| <i>S. exigua</i>     | Lep535-1          | 39.9                             |
|                      | Lep527-1          | 57.5                             |
|                      | Lep529-1          | 52.8                             |
|                      | Lep539-1          | 140.8                            |
|                      | Lep540-1          | 59.9                             |
|                      | Lep565-1          | 102.1                            |

**Supplementary Table S2.** Information on voucher ID, instar stage, collecting locality, GPS coordinates, code in each figure, and accession numbers of noctuid moths.

Sequences downloaded from GenBank are listed below the dashed line

| Taxon                        | Voucher ID | Instar stage          | Collecting locality                                       | GPS-E       | GPS-N       | Code in this study |        |        |        |         |         |         |         |         |          | Accession No. |
|------------------------------|------------|-----------------------|-----------------------------------------------------------|-------------|-------------|--------------------|--------|--------|--------|---------|---------|---------|---------|---------|----------|---------------|
|                              |            |                       |                                                           |             |             | Fig. 1             | Fig. 2 | Fig. 3 | Fig. 4 | Fig. S2 | Fig. S3 | Fig. S4 | Fig. S5 | Fig. S6 |          |               |
| <i>Euproctis taiwana</i>     | Lep76-1    | Adult                 | Fuchih Farm, Gukeng Township, Yunlin County, Taiwan       | 120.534811° | 23.64085°   |                    | 6      |        |        |         | 6       |         |         |         | -        |               |
|                              | Lep80-1    | Adult                 | National Chiayi University, Taiwan                        | 23.473190°  | 120.485723° |                    | 7      |        |        |         | 7       |         |         |         | -        |               |
| <i>Helicoverpa armigera</i>  | Lep66-1    | Unknown               | National Chiayi University, Taiwan                        | 23.473190°  | 120.485723° |                    | 8      |        |        |         | 8       |         |         |         | -        |               |
|                              | Lep495-1   | 3 <sup>rd</sup> larva | Thailand                                                  | -           | -           |                    | 9      |        |        |         | 9       |         |         |         | -        |               |
| <i>Hydrillodes lentalis</i>  | Lep580-3   | 4 <sup>th</sup> larva | Fenyuan Township, Changhua County, Taiwan                 | -           | -           |                    |        |        | 3      |         |         |         | 3       |         | LC508666 |               |
|                              | Lep582-1   | 5 <sup>th</sup> larva | Wanggong, Fangyuan Township, Chuang County, Taiwan        | 23.961990°  | 120.338150° |                    | 10     |        |        |         | 10      |         | 5       |         | LC508669 |               |
| <i>Mythimna separata</i>     | Lep582-2   | 5 <sup>th</sup> larva | Wanggong, Fangyuan Township, Chuang County, Taiwan        | 23.961990°  | 120.338150° |                    | 11     |        | 6      |         | 11      |         | 6       |         | LC508667 |               |
|                              | Lep582-3   | 5 <sup>th</sup> larva | Wanggong, Fangyuan Township, Chuang County, Taiwan        | 23.961990°  | 20.338150°  |                    | 12     |        | 7      |         | 12      |         | 7       |         | LC508668 |               |
| <i>Ostrinia furnacalis</i>   | Lep87-1    | Adult                 | Fuchih Farm, Gukeng Township, Yunlin County, Taiwan       | 23.64085°   | 120.534811° |                    | 13     |        |        |         | 13      |         |         |         | -        |               |
|                              | Lep87-2    | Adult                 | Fuchih Farm, Gukeng Township, Yunlin County, Taiwan       | 23.64085°   | 120.534811° |                    | 14     |        |        |         | 14      |         |         |         | -        |               |
|                              | Lep588-1   | 5 <sup>th</sup> larva | Guoyesinduan, Husi Township, Penghu County, Taiwan        | 23.576453°  | 119.679679° |                    |        |        | 17     |         |         |         | 17      |         | LC508670 |               |
|                              | Lep588-2   | 5 <sup>th</sup> larva | Guoyesinduan, Husi Township, Penghu County, Taiwan        | 23.576453°  | 119.679679° |                    |        |        | 18     |         |         |         | 18      |         | LC508671 |               |
|                              | Lep588-3   | 5 <sup>th</sup> larva | Guoyesinduan, Husi Township, Penghu County, Taiwan        | 23.576453°  | 119.679679° |                    |        |        | 19     |         |         |         | 19      |         | LC508672 |               |
| <i>Simplicia cornicalis</i>  | Lep580-1   | 5 <sup>th</sup> larva | Fenyuan Township, Changhua County, Taiwan                 | -           | -           |                    |        |        | 1      |         |         |         | 1       |         | LC508673 |               |
|                              | Lep580-2   | 5 <sup>th</sup> larva | Fenyuan Township, Changhua County, Taiwan                 | -           | -           |                    |        |        | 2      |         |         |         | 2       |         | LC508674 |               |
|                              | Lep580-4   | 5 <sup>th</sup> larva | Fenyuan Township, Changhua County, Taiwan                 | -           | -           |                    |        |        | 4      |         |         |         | 4       |         | LC508675 |               |
| <i>Spoladea recurvalis</i>   | Lep74-1    | Adult                 | Fuchih Farm, Gukeng Township, Yunlin County               | 120.534811° | 23.64085°   |                    | 15     |        |        |         | 15      |         |         |         | -        |               |
|                              | Lep89-1    | Adult                 | Fuchih Farm, Gukeng Township, Yunlin County               | 120.534811° | 23.64085°   |                    | 16     |        |        |         | 16      |         |         |         | -        |               |
| <i>Spodoptera frugiperda</i> | Lep581-2   | 5 <sup>th</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           | 1                  | 1      |        |        | 1       | 1       | 17      |         |         | LC508676 |               |
|                              | Lep581-3   | 6 <sup>th</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           | 2                  | 2      |        |        | 2       | 2       | 18      |         |         | -        |               |
|                              | Lep581-4   | egg                   | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 19      |         | 7       | -        |               |
|                              | Lep581-5   | egg                   | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 20      |         | 8       | -        |               |
|                              | Lep581-6   | 1 <sup>st</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 21      |         | 9       | -        |               |
|                              | Lep581-7   | 1 <sup>st</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 22      |         | 10      | -        |               |
|                              | Lep581-8   | 2 <sup>nd</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 23      |         | 11      | -        |               |
|                              | Lep581-9   | 2 <sup>nd</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 24      |         |         | -        |               |
|                              | Lep581-10  | 2 <sup>nd</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 25      |         |         | -        |               |
|                              | Lep581-11  | 2 <sup>nd</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 26      |         |         | -        |               |
|                              | Lep581-12  | -                     | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 27      |         |         | -        |               |
|                              | Lep581-13  | 3 <sup>rd</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 28      |         |         | -        |               |
|                              | Lep581-14  | 4 <sup>th</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 29      |         |         | -        |               |
|                              | Lep581-15  | 5 <sup>th</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 30      |         |         | -        |               |
|                              | Lep581-16  | 5 <sup>th</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 31      |         |         | -        |               |
|                              | Lep581-17  | 6 <sup>th</sup> larva | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 32      |         |         | -        |               |
|                              | Lep581-18  | Pupa                  | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 33      |         |         | -        |               |
|                              | Lep581-19  | Pupa                  | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 34      |         |         | -        |               |
|                              | Lep581-20  | Pupa                  | Department of Entomology, University of Pennsylvania, USA | -           | -           |                    |        |        |        |         |         | 35      |         |         | -        |               |
|                              | Lep582-4   | 6 <sup>th</sup> larva | Wanggong, Fangyuan Township, Chuang County, Taiwan        | 23.961990°  | 120.338150° |                    |        |        | 1      |         |         | 1       |         | 8       | LC508677 |               |
|                              | Lep583-1   | 6 <sup>th</sup> larva | Cingshuei Township, Taichung City, Taiwan                 | -           | -           | 3                  | 3      | 2      | 9      | 3       | 3       | 2       |         | 9       | -        |               |
|                              | Lep583-2   | 6 <sup>th</sup> larva | Cingshuei Township, Taichung City, Taiwan                 | -           | -           | 4                  |        | 3      | 10     | 4       |         | 3       |         | 10      | LC508678 |               |
|                              | Lep583-3   | 6 <sup>th</sup> larva | Cingshuei Township, Taichung City, Taiwan                 | -           | -           |                    |        | 4      | 11     |         |         | 4       |         | 11      | LC508679 |               |
|                              | Lep583-4   | 5 <sup>th</sup> larva | Cingshuei Township, Taichung City, Taiwan                 | -           | -           |                    |        | 5      | 12     |         |         | 5       |         | 12      | LC508680 |               |
|                              | Lep584-1   | 6 <sup>th</sup> larva | Sihu Township, Changhua County, Taiwan                    | -           | -           | 5                  |        | 6      | 13     | 5       |         | 6       |         | 13      | LC508681 |               |
|                              | Lep584-2   | 5 <sup>th</sup> larva | Sihu Township, Changhua County, Taiwan                    | -           | -           | 6                  |        | 7      | 14     | 6       |         | 7       |         | 14      | LC508682 |               |

Supplementary Table S2 (continued)

| Taxon                        | Voucher ID            | Larval stage          | Collecting Locality                                | GPS-E      | GPS-N       | Code in this study |        |        |        |         |         |         |         |         |          | Accession No. |
|------------------------------|-----------------------|-----------------------|----------------------------------------------------|------------|-------------|--------------------|--------|--------|--------|---------|---------|---------|---------|---------|----------|---------------|
|                              |                       |                       |                                                    |            |             | Fig. 1             | Fig. 2 | Fig. 3 | Fig. 4 | Fig. S2 | Fig. S3 | Fig. S4 | Fig. S5 | Fig. S6 |          |               |
| <i>Spodoptera frugiperda</i> | Lep584-3              | 6 <sup>th</sup> larva | Sihu Township, Changhua County, Taiwan             | -          | -           |                    |        | 8      | 15     |         |         | 8       | 15      | 1       | LC508683 |               |
|                              | Lep584-4              | 6 <sup>th</sup> larva | Sihu Township, Changhua County, Taiwan             | -          | -           |                    |        | 9      | 16     |         |         | 9       | 16      | 2       | LC508684 |               |
|                              | Lep589-1              | 6 <sup>th</sup> larva | Guoyesinduan, Husi Township, Penghu County, Taiwan | 23.576453° | 119.679679° |                    |        | 10     | 20     |         |         | 10      | 20      | 3       | LC508685 |               |
|                              | Lep590-1              | 6 <sup>th</sup> larva | Sisisinduan, Penghu County, Taiwan                 | 23.581579° | 119.638308° |                    |        | 11     | 21     |         |         | 11      | 21      |         | LC508686 |               |
|                              | Lep590-2              | 6 <sup>th</sup> larva | Sisisinduan, Penghu County, Taiwan                 | 23.581579° | 119.638308° |                    |        | 12     | 22     |         |         | 12      | 22      |         | LC508687 |               |
|                              | Lep591-1              | 4 <sup>th</sup> larva | Taiwu siduan, Penghu County, Taiwan                | 23.571097° | 119.635640° |                    |        | 13     | 23     |         |         | 13      | 23      |         | LC508688 |               |
|                              | Lep591-2              | Adult                 | Taiwu siduan, Penghu County, Taiwan                | 23.571097° | 119.635640° |                    |        | 14     | 24     |         |         | 14      | 24      |         | LC508689 |               |
|                              | Lep592-1              | 6 <sup>th</sup> larva | Checheng, Pingtung County, Taiwan                  | -          | -           |                    |        | 15     | 25     |         |         | 15      | 25      | 4       | LC508690 |               |
|                              | Lep592-2              | 6 <sup>th</sup> larva | Checheng, Pingtung County, Taiwan                  | -          | -           |                    |        | 16     | 26     |         |         | 16      | 26      |         | LC508691 |               |
|                              | Lep592-3              | 6 <sup>th</sup> larva | Checheng, Pingtung County, Taiwan                  | -          | -           |                    |        |        | 27     |         |         | 49      | 27      |         | LC508692 |               |
|                              | Lep592-4              | 6 <sup>th</sup> larva | Checheng, Pingtung County, Taiwan                  | -          | -           |                    |        |        | 28     |         |         | 50      | 28      |         | LC508693 |               |
|                              | Lep592-5              | 6 <sup>th</sup> larva | Checheng, Pingtung County, Taiwan                  | -          | -           |                    |        |        | 29     |         |         | 51      | 29      |         | LC508694 |               |
|                              | Lep652-1              | 6 <sup>th</sup> larva | Houli District, Taichung City, Taiwan              | 24.315566° | 120.715018° |                    |        |        |        |         |         | 52      |         |         | LC508695 |               |
|                              | Lep652-2              | 6 <sup>th</sup> larva | Houli District, Taichung City, Taiwan              | 24.315566° | 120.715018° |                    |        |        |        |         |         | 53      |         |         | LC508696 |               |
|                              | Lep670-1              | 6 <sup>th</sup> larva | Mituo, Kaohsiung City, Taiwan                      | 22.773713° | 120.248315° |                    |        |        |        |         |         | 54      |         |         | LC508697 |               |
|                              | Lep670-2              | 6 <sup>th</sup> larva | Mituo, Kaohsiung City, Taiwan                      | 22.773713° | 120.248315° |                    |        |        |        |         |         | 55      |         |         | LC508698 |               |
|                              | Lep670-3              | 6 <sup>th</sup> larva | Mituo, Kaohsiung City, Taiwan                      | 22.773713° | 120.248315° |                    |        |        |        |         |         | 56      |         |         | LC508699 |               |
|                              | Lep682-1              | 6 <sup>th</sup> larva | Luona, Sinyi Township, Nantou County, Taiwan       | 23.630372° | 120.880075° |                    |        |        |        |         |         | 57      |         |         | LC508700 |               |
|                              | Lep683-1              | 4 <sup>th</sup> larva | Annan District, Tainan City                        | 23.071835° | 120.190285° |                    |        |        |        |         |         | 58      |         |         | -        |               |
|                              | Lep683-2              | 6 <sup>th</sup> larva | Annan District, Tainan City                        | 23.071835° | 120.190285° |                    |        |        |        |         |         | 59      |         | 5       | LC508701 |               |
|                              | Lep683-3              | 6 <sup>th</sup> larva | Annan District, Tainan City                        | 23.071835° | 120.190285° |                    |        |        |        |         |         | 60      |         | 6       | LC508702 |               |
|                              | Lep683-4              | 6 <sup>th</sup> larva | Annan District, Tainan City                        | 23.071835° | 120.190285° |                    |        |        |        |         |         | 61      |         |         | LC508703 |               |
|                              | Lep683-5              | 6 <sup>th</sup> larva | Annan District, Tainan City                        | 23.071835° | 120.190285° |                    |        |        |        |         |         | 62      |         |         | LC508704 |               |
|                              | Lep683-6              | 6 <sup>th</sup> larva | Annan District, Tainan City                        | 23.071835° | 120.190285° |                    |        |        |        |         |         | 63      |         |         | LC508705 |               |
|                              | Lep752-1              | 6 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 36      |         | 12      | -        |               |
|                              | Lep752-2              | 6 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 37      |         | 13      | -        |               |
|                              | Lep752-3              | 6 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 38      |         | 14      | -        |               |
|                              | Lep752-4              | 6 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 39      |         | 15      | -        |               |
|                              | Lep752-5              | 4 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 40      |         | 16      | -        |               |
|                              | Lep752-6              | 5 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 41      |         |         | -        |               |
|                              | Lep752-7              | 4 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 42      |         |         | -        |               |
|                              | Lep752-8              | 4 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 43      |         |         | -        |               |
|                              | Lep752-9              | 4 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 44      |         |         | -        |               |
|                              | Lep752-10             | 4 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 45      |         |         | -        |               |
|                              | Lep752-11             | 5 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 46      |         |         | -        |               |
|                              | Lep752-12             | 4 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 47      |         |         | -        |               |
|                              | Lep752-13             | 4 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 48      |         |         | -        |               |
|                              | Lep752-14             | 5 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 64      |         |         | -        |               |
|                              | Lep752-15             | 4 <sup>th</sup> larva | Thailand                                           | -          | -           |                    |        |        |        |         |         | 65      |         |         | -        |               |
| Lep752-16                    | 4 <sup>th</sup> larva | Thailand              | -                                                  | -          |             |                    |        |        |        |         | 66      |         |         | -       |          |               |
| Lep752-17                    | 4 <sup>th</sup> larva | Thailand              | -                                                  | -          |             |                    |        |        |        |         | 67      |         |         | -       |          |               |
| Lep752-18                    | 4 <sup>th</sup> larva | Thailand              | -                                                  | -          |             |                    |        |        |        |         | 68      |         |         | -       |          |               |
| Lep752-19                    | 2 <sup>nd</sup> larva | Thailand              | -                                                  | -          |             |                    |        |        |        |         | 69      |         |         | -       |          |               |
| <i>S. litura</i>             | Lep65-1               | Adult                 | National Chiayi University, Taiwan                 | 23.473190° | 120.485723° | 7                  |        |        |        | 7       |         |         |         |         | -        |               |
|                              | Lep65-2               | Adult                 | National Chiayi University, Taiwan                 | 23.473190° | 120.485723° | 8                  |        |        |        | 8       |         |         |         |         | -        |               |
|                              | Lep191-1              | Adult                 | Nanao Township, Yilan County, Taiwan               | 24.423041° | 121.789871° | 9                  |        |        |        | 9       |         |         |         |         | -        |               |

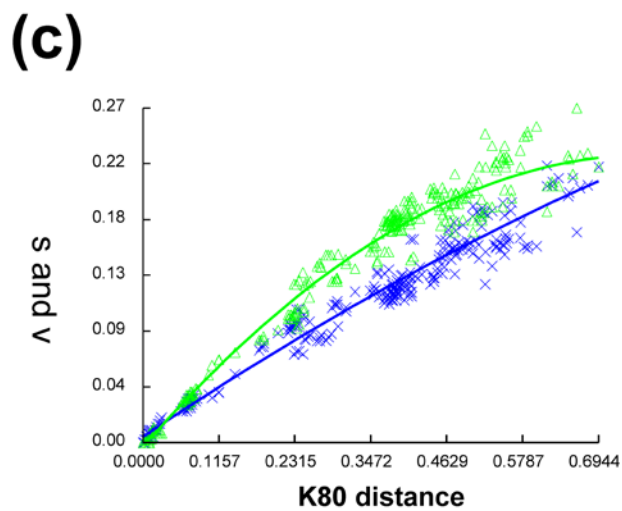
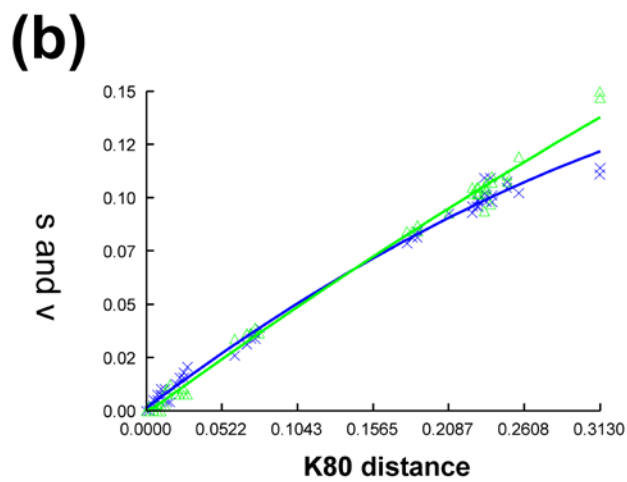
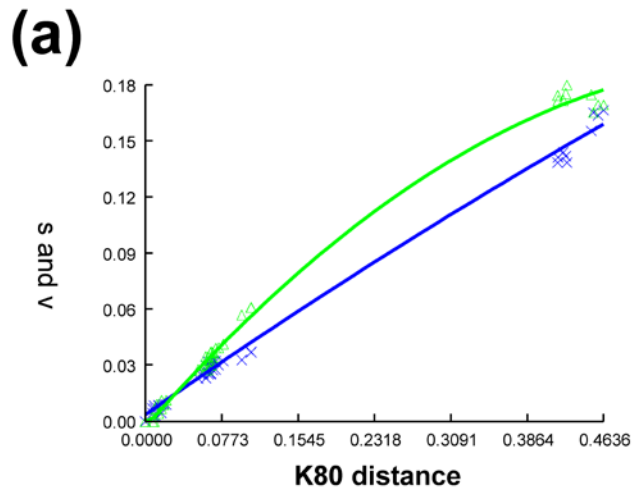
Supplementary Table S2 (continued)

| Taxon                       | Voucher ID | Larval stage          | Collecting Locality | GPS-E | GPS-N | Code in this study |        |        |        |         |         |         |         |         |          | Accession No. |
|-----------------------------|------------|-----------------------|---------------------|-------|-------|--------------------|--------|--------|--------|---------|---------|---------|---------|---------|----------|---------------|
|                             |            |                       |                     |       |       | Fig. 1             | Fig. 2 | Fig. 3 | Fig. 4 | Fig. S2 | Fig. S3 | Fig. S4 | Fig. S5 | Fig. S6 |          |               |
| <i>S. litura</i>            | Lep528-1   | 2 <sup>nd</sup> larva | Thailand            | -     | -     |                    |        | 10     |        |         |         | 10      |         |         | -        |               |
|                             | Lep535-1   | 1 <sup>st</sup> larva | Thailand            | -     | -     |                    |        | 11     |        |         |         | 11      |         |         | -        |               |
| <i>S. exigua</i>            | Lep527-1   | 2 <sup>nd</sup> larva | Thailand            | -     | -     |                    |        | 12     |        |         |         | 12      |         |         | -        |               |
|                             | Lep529-1   | 2 <sup>nd</sup> larva | Thailand            | -     | -     |                    |        | 13     | 4      |         |         | 13      |         |         | -        |               |
|                             | Lep539-1   | 2 <sup>nd</sup> larva | Thailand            | -     | -     |                    |        | 14     | 5      |         |         | 14      |         |         | -        |               |
|                             | Lep540-1   | 3 <sup>rd</sup> larva | Thailand            | -     | -     |                    |        | 15     |        |         |         | 15      |         |         | -        |               |
|                             | Lep565-1   | 3 <sup>rd</sup> larva | Thailand            | -     | -     |                    |        | 16     |        |         |         | 16      |         |         | -        |               |
| <i>Helicoverpa armigera</i> | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | EU204143 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | KT343377 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | KT343378 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | AJ577253 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | AB620127 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | AF401740 |               |
| <i>Helicoverpa zea</i>      | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | KT343375 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | KT343376 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | KT343380 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | KT343381 |               |
| <i>Helicoverpa assulta</i>  | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | KT343382 |               |
| <i>Heliothis subflexa</i>   | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | KT762150 |               |
| <i>Heliothis virescens</i>  | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | KT343379 |               |
| <i>Mythimna separata</i>    | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | LC361446 |               |
| <i>S. exigua</i>            | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | JN863291 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | JN863292 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | JN863293 |               |
| <i>S. litura</i>            | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | FJ041111 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | JN863294 |               |
| <i>S. frugiperda</i>        | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | GQ478352 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779574 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779575 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779576 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779577 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779578 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779579 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779580 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779581 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779582 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779583 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779584 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH779585 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH819372 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH819373 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH819374 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH819375 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH819376 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH819377 |               |
|                             | -          | -                     | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH819378 |               |

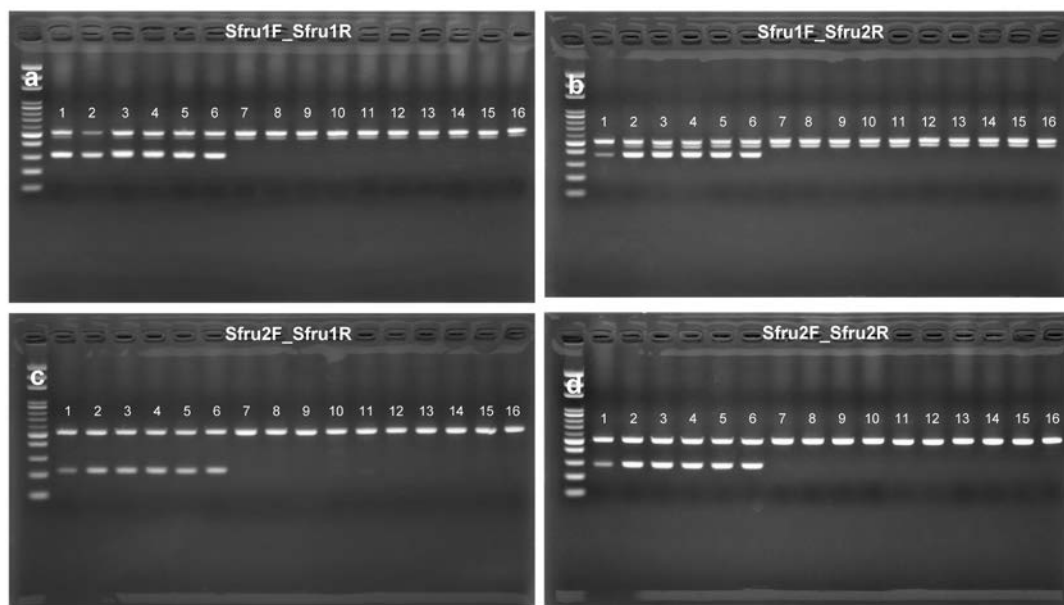
Supplementary Table S2 (continued)

| Taxon                | Voucher ID | Larval stage | Collecting Locality | GPS-E | GPS-N | Code in this study |        |        |        |         |         |         |         |         |          | Accession No. |
|----------------------|------------|--------------|---------------------|-------|-------|--------------------|--------|--------|--------|---------|---------|---------|---------|---------|----------|---------------|
|                      |            |              |                     |       |       | Fig. 1             | Fig. 2 | Fig. 3 | Fig. 4 | Fig. S2 | Fig. S3 | Fig. S4 | Fig. S5 | Fig. S6 |          |               |
| <i>S. frugiperda</i> | -          | -            | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH819379 |               |
|                      | -          | -            | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH819380 |               |
|                      | -          | -            | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | MH819381 |               |
|                      | -          | -            | -                   | -     | -     | -                  | -      | -      | -      | -       | -       | -       | -       | -       | HM851175 |               |

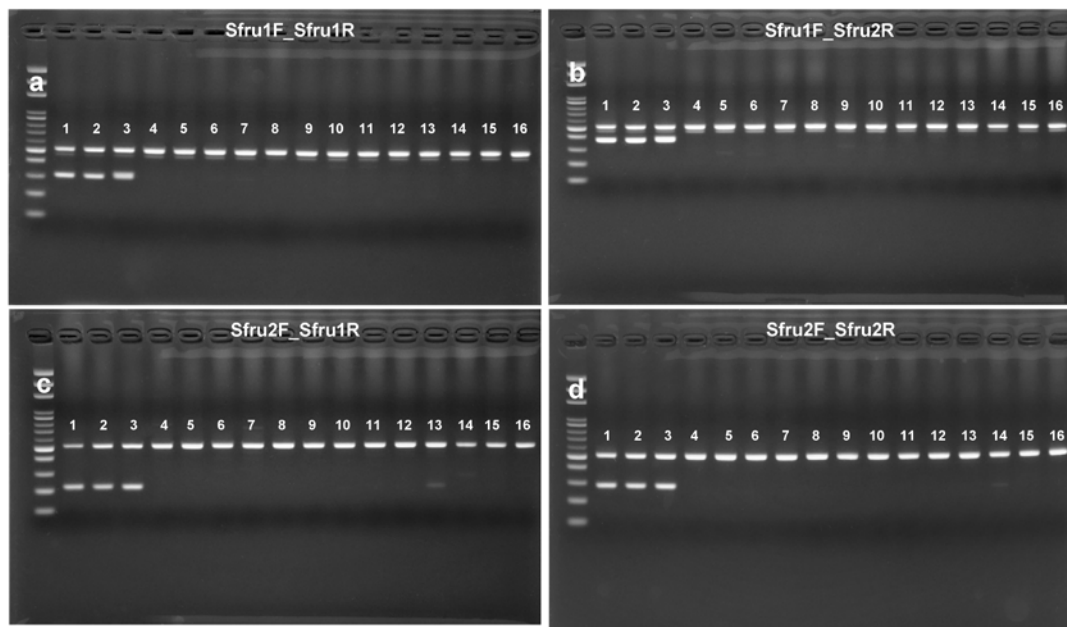
**Supplementary Figure S1.** Plot distribution of ITS1 sequence variations in categories of genus *Helicoverpa* **(a)**, genus *Spodoptera* **(b)**, and Noctuidae **(c)**.



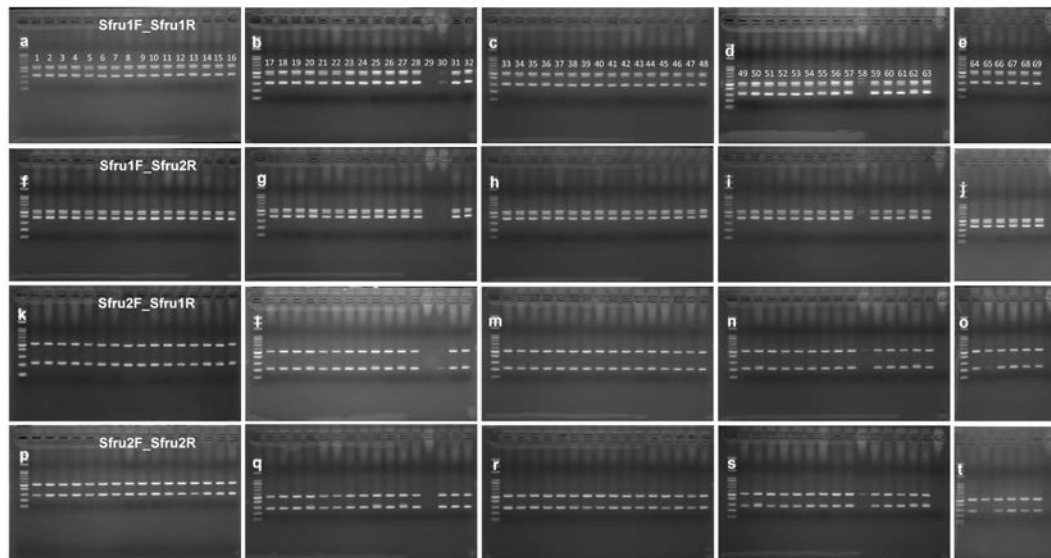
**Supplementary Figure S2.** Multiplex PCR using a FAW-specific primer set with universal paired primers (uncropped gel). A specific fragment is visible in target moths with no cross-amplification. The 100-bp DNA ladder and specific primer sets of Sfru1F\_Sfru1R **(a)**, Sfru1F\_Sfru2R **(b)**, Sfru2F\_Sfru1R **(c)**, and Sfru2F\_Sfru2R **(d)** are displayed on each panel. Lanes 1–6, FAW; 7–11, *Spodoptera litura*; and 12–16, *Spodoptera exigua*. Pertinent information of each individual of each lane is presented in **Supplementary Table S2**.



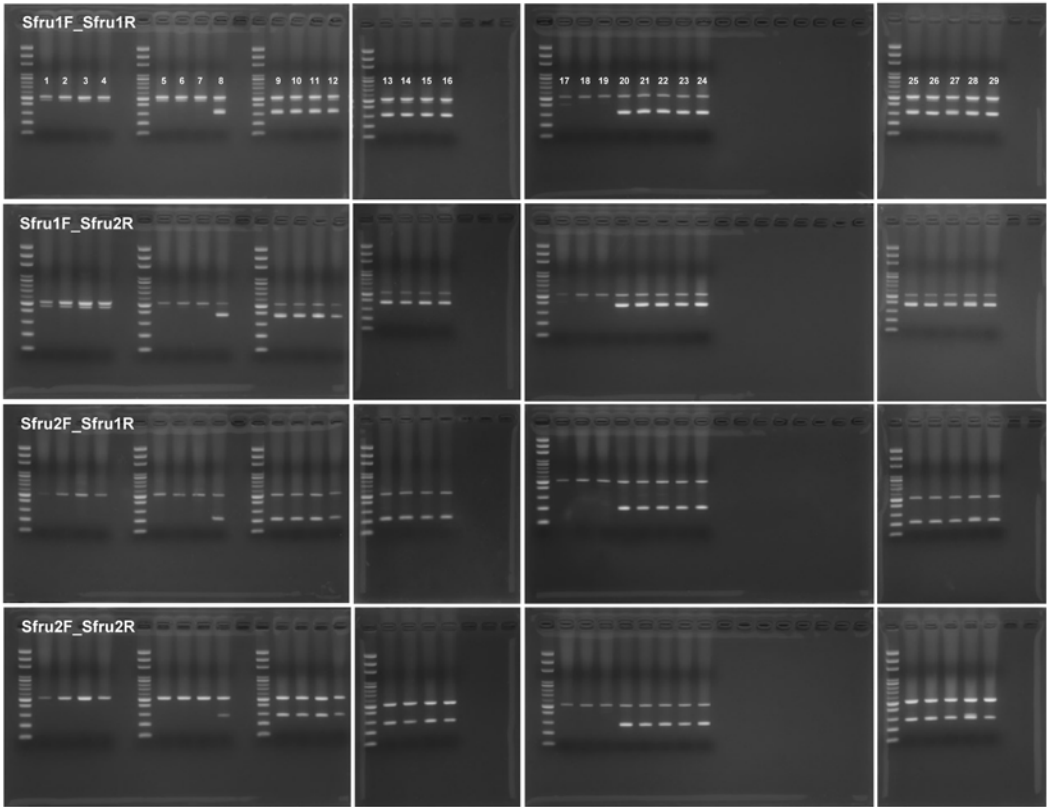
**Supplementary Figure S3.** Stability test of a FAW-specific primer set with universal paired primers among the eight corn pests in the field (uncropped gel). Specific amplification fragment is visible in target moths with no cross-amplification. The 100-bp DNA ladder and specific primer sets of Sfru1F\_Sfru1R **(a)**, Sfru1F\_Sfru2R **(b)**, Sfru2F\_Sfru1R **(c)**, and Sfru2F\_Sfru2R **(d)** are displayed on each panel. Lanes 1–3, FAW; 4 and 5, *Spodoptera exigua*; 6 and 7, *Euproctis taiwana*; 8 and 9, *Helicoverpa armigera*; 10, *Mythimna separate*; 11 and 12, *Mythimna loreyi*; 13 and 14, *Ostrinia furnacalis*; and 15 and 16, *Spoladea recurvalis*. Pertinent information of each individual of each lane is given in **Supplementary Table S2**.



**Supplementary Figure S4.** Efficacy test of a FAW-specific primer set with universal paired primers of partial FAW samples (uncropped gel). The 100-bp DNA ladder and specific primer sets of Sfru1F\_Sfru1R (**a–e**), Sfru1F\_Sfru2R (**f–j**), Sfru2F\_Sfru1R (**k–o**), and Sfru2F\_Sfru2R (**p–t**), are displayed on each panel. Pertinent information on each individual of each lane is presented in **Supplementary Table S2**.



**Supplementary Figure S5.** Field sample detection by using FAW-specific primer pairs of Sfru1F\_Sfru1R, Sfru1F\_Sfru2R, Sfru2F\_Sfru1R, and Sfru2F\_Sfru2R (uncropped gel). The 100-bp DNA ladder is shown on the left lane. Pertinent information of each individual of each lane is presented in **Supplementary Table S2**.



**Supplementary Figure S6.** FAWs DNA dilution test of 1X (**a—d**), 10X (**e—h**), 100X (**i—l**), and 1,000X (**m—p**) using FAW-specific primer pairs of Sfru1F\_Sfru1R, Sfru1F\_Sfru2R, Sfru2F\_Sfru1R, and Sfru2F\_Sfru2R (uncropped gel). The 100-bp DNA ladder is shown. Pertinent information of each individual of each lane is presented in **Supplementary Table S2**.

